BICYCLO-PYRAZOLES ACTIVE AS KINASE INHIBITORS, PROCESS FOR THEIR PREPARATION AND PHARMACEUTICAL COMPOSITIONS COMPRISING THEM

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to bicyclo-pyrazole derivatives active as kinase inhibitors and, more in particular, it relates to pyrazole-tetrahydro pyridine derivatives, to a process for their preparation, to pharmaceutical compositions comprising them and to their use as therapeutic agents, particularly in the treatment of diseases linked to disregulated protein kinases.

Discussion of the Background

The malfunctioning of protein kinases (PKs) is the hallmark of numerous diseases.

A large share of the oncogenes and proto-oncogenes involved in human cancers code for PKs. The enhanced activities of PKs are also implicated in many non-malignant diseases such as benign prostate hyperplasia, familial adenomatosis, polyposis, neuro-fibromatosis, psoriasis, vascular smooth cell proliferation associated with atherosclerosis, pulmonary fibrosis, arthritis glomerulonephritis and post-surgical stenosis and restenosis.

PKs are also implicated in inflammatory conditions and in the multiplication of viruses and parasites. PKs may also play a major role in the pathogenesis and development of neurodegenerative disorders.

For a general reference to PKs malfunctioning or disregulation see, for instance, Current Opinion in Chemical Biology 1999, 3, 459-465.

Some pyrazole-tetrahydro pyridine derivative are known in the art.

Few pyrazole-tetrahydro pyridine derivatives were studied as inhibitors of phosphodiesterase and TNF production, see WO95/01980A1 and WO96/12720; of farnesyl protein transferase in CA2,143,588; of PDE V in WO00/119802.

Some pyrazole-tetrahydro pyridine derivatives with anti-inflammatory activity were disclosed in FR1,463,883; and the activity of some other pyrazole-tetrahydro pyridine derivatives in the CNS or cardiovascular therapeutic field was shown in WO97/32848, WO99/33804, US4,500,525, US6,187,774 and US6,265,418.

SUMMARY OF THE INVENTION

The present inventors have now discovered that some pyrazole-tetrahydro pyridines are endowed with multiple protein kinase inhibiting activity and are thus useful in therapy in the treatment of diseases caused by and/or associated with disregulated protein kinases.

As such, it is an object of the invention to provide compounds which are useful as therapeutic agents against a host of diseases caused by a disregulated protein kinase activity.

It is another object to provide compounds endowed with multiple protein kinase inhibiting activity.

More specifically, the pyrazole-tetrahydro pyridines of this invention are useful in the treatment of a variety of cancers including, but not limited to: carcinoma such as bladder, breast, colon, kidney, liver, lung, including small cell lung cancer, esophagus, gall-bladder, ovary, pancreas, stomach, cervix, thyroid, prostate, and skin, including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocitic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell-lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma and Burkett's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias, myelodysplastic syndrome and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma and schwannomas; other tumors, including melanoma, seminoma, teratocarcinoma, osteosarcoma, xeroderma pigmentosum, keratocanthoma, thyroid follicular cancer and Kaposi's sarcoma.

Due to the key role of PKs in the regulation of cellular proliferation, these pyrazole-tetrahydro pyridines are also useful in the treatment of a variety of cell proliferative disorders such as, for instance, benign prostate hyperplasia, familial adenomatosis,

polyposis, neuro-fibromatosis, psoriasis, vascular smooth cell proliferation associated with atherosclerosis, pulmonary fibrosis, arthritis glomerulonephritis and post-surgical stenosis and restenosis.

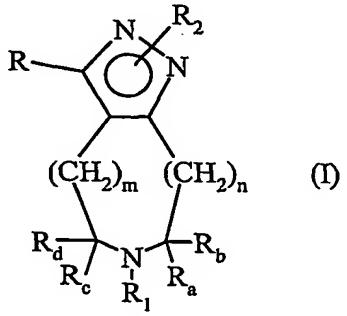
The compounds of the invention can be useful in the treatment of Alzheimer's disease, as suggested by the fact that cdk5 is involved in the phosphorylation of tau protein (J. Biochem., 117, 741-749, 1995).

The compounds of this invention, as modulators of apoptosis, may also be useful in the treatment of cancer, viral infections, prevention of AIDS development in HIV-infected individuals, autoimmune diseases and neurodegenerative disorders.

The compounds of this invention may be useful in inhibiting tumor angiogenesis and metastasis.

The compounds of the invention are useful as cyclin dependent kinase (cdk) inhibitors and also as inhibitors of other protein kinases such as, for instance, protein kinase C in different isoforms, Met, PAK-4, PAK-5, ZC-1, STLK-2, DDR-2, Aurora 1, Aurora 2, Bub-1, PLK, Chk1, Chk2, HER2, raf1, MEK1, MAPK, EGF-R, PDGF-R, FGF-R, IGF-R, VEGF-R, PI3K, weel kinase, Src, Abl, Akt, ILK, MK-2, IKK-2, Cdc7, Nek, and thus be effective in the treatment of diseases associated with other protein kinases.

Accordingly, the present invention provides a method for treating diseases caused by and/or associated with an altered protein kinase activity which comprises administering to a mammal in need thereof an effective amount of a pyrazole-tetrahydro pyridine derivative represented by formula (I):



(I)

wherein R represents hydrogen or halogen atom, or an optionally substituted group selected from aryl C_2-C_6 alkenyl, (heterocyclyl) C_2-C_6 alkenyl,

C_2-C_6 alkynyl, aryl C_2-C_6 alkynyl, or (heterocyclyl) C_2-C_6 alkynyl group, -R', -COR',

-COOR', -CN, -CONR'R", -OR', -$S(O)_qR'$, -$SO_2NR'R"$, -$B(OR"')_2$, -SnR"", wherein R' and R", the same or different, independently represent hydrogen atom or an optionally further substituted straight or branched C_1-C_6 alkyl, C_2-C_6 alkenyl, saturated or unsaturated C_3-C_6 cycloalkyl, aryl, heterocyclyl, aryl C_1-C_6 alkyl or (heterocyclyl)C_1-C_6 alkyl; R"' represents hydrogen, C_1-C_6 alkyl, or R"', together with the two oxygen and the boron atoms, forms a saturated or unsaturated C_5-C_8 (hetero)cycloalkyl, optionally benzocondensed or substituted, and R"" represents C_1-C_6 alkyl;

R_1 represents hydrogen atom or an optionally substituted group selected from –R', -CH_2R', -COR', -COOR', -CONR'R", -NH-C(=NH)NHR', -C(=NH)NHR', -$S(O)_qR'$, or -$SO_2NR'R"$, wherein R' and R" are as defined above;

R_2 represents hydrogen atom, -COR', -COOR', -CONR'R", -$S(O)_q$ R', -$SO_2NR'R"$, C_1-C_6 alkyl or (heterocyclyl)C_1-C_6 alkyl group, wherein R' and R" are as defined above;

R_a, R_b, R_c and R_d, being the same or different, independently represent hydrogen atom, an optionally further substituted straight or branched C_1-C_6 alkyl, aryl, heterocyclyl, aryl C_1-C_6 alkyl, (heterocyclyl)C_1-C_6 alkyl or -CH_2OR' group, wherein R' is as above defined, or R_a and R_b and/or R_c and R_d, taken together with the carbon atom to which they are bonded, form an optionally substituted, saturated or unsaturated, C_3-C_6 cycloalkyl group; q is 0, 1 or 2; m and n, each independently, represents 0 or 1, provided that m + n is equal to 1; or a pharmaceutically acceptable salt thereof.

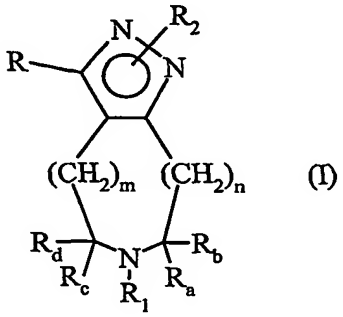
In a preferred embodiment of the method described above, the disease caused by and/or associated with an altered protein kinase activity is selected from the group consisting of cancer, cell proliferative disorders, Alzheimer's disease, viral infections, auto-immune diseases and neurodegenerative disorders.

Specific types of cancer that may be treated according to the invention include carcinoma, squamous cell carcinoma, hematopoietic tumors of myeloid or lymphoid lineage, tumors of mesenchymal origin, tumors of the central and peripheral nervous system, melanoma, seminoma, teratocarcinoma, osteosarcoma, xeroderoma pigmentosum, keratoxanthoma, thyroid follicular cancer and Kaposi's sarcoma.

In another preferred embodiment of the method described above, the cell proliferative disorder is selected from the group consisting of benign prostate hyperplasia, familial

adenomatosis polyposis, neuro-fibromatosis, psoriasis, vascular smooth cell proliferation associated with atherosclerosis, pulmonary fibrosis, arthritis glomerulonephritis and post-surgical stenosis and restenosis. In addition, the method object of the present invention, provides tumor angiogenesis and metastasis inhibition.

The present invention also provides a pyrazole-tetrahydro pyridine derivative represented by formula (I):



(I)

wherein R represents hydrogen or halogen atom, or an optionally substituted group selected from aryl C_2-C_6 alkenyl, (heterocyclyl) C_2-C_6 alkenyl, C_2-C_6 alkynyl, aryl C_2-C_6 alkynyl, or (heterocyclyl) C_2-C_6 alkynyl group, -R', -COR', -COOR', -CN, -CONR'R", -OR', -$S(O)_qR'$, -$SO_2NR'R"$, -$B(OR"')_2$, -SnR"", wherein R' and R", the same or different, independently represent hydrogen atom or an optionally further substituted straight or branched C_1-C_6 alkyl, C_2-C_6 alkenyl, saturated or unsaturated C_3-C_6 cycloalkyl, aryl, heterocyclyl, aryl C_1-C_6 alkyl or (heterocyclyl)C_1-C_6 alkyl; R"' represents hydrogen, C_1-C_6 alkyl, or R"', together with the two oxygen and the boron atoms, forms a saturated or unsaturated C_5-C_8 (hetero)cycloalkyl, optionally benzocondensed or substituted, and R"" represents C_1-C_6 alkyl;

R_1 represents hydrogen atom or an optionally substituted group selected from –R', -CH_2R',-COR', -COOR', -CONR'R", -NH-C(=NH)NHR', -C(=NH)NHR', -$S(O)_qR'$, or -$SO_2NR'R"$, wherein R' and R" are as defined above;

R_2 represents hydrogen atom, -COR', -COOR', -CONR'R", -$S(O)_q$ R', -$SO_2NR'R"$, C_1-C_6 alkyl or (heterocyclyl)C_1-C_6 alkyl group, wherein R' and R" are as defined above;

- R_a, R_b, R_c and R_d, being the same or different, independently represent hydrogen atom, an optionally further substituted straight or branched C_1-C_6 alkyl, aryl, heterocyclyl,

  aryl C_1-C_6 alkyl, (heterocyclyl)C_1-C_6 alkyl or -CH_2OR' group, wherein R' is as above defined, or R_a and R_b and/or R_c and R_d, taken together with the carbon atom to which they are bonded, form an optionally substituted, saturated or unsaturated, C_3-C_6 cycloalkyl group; q is 0, 1 or 2; m and n, each independently, represents 0 or 1, provided that m + n is equal to 1; with the following further provisos:

- when m is 0 and n is 1, R_2 is hydrogen, R_a, R_b, R_c and R_d are hydrogen atoms or methyl groups, and R is hydrogen atom, hydroxy or methyl group, then R_1 is not hydrogen atom or methyl, benzyl, t-BOC, pyrimidyl, tetrahydrobenzindole, quinolinecarboxy, pyridobenzoxazino or naphtyridino group;
- when m is 0 and n is 1, R is an optionally substituted phenyl group, furanyl, thienyl, or carboxyethyl, and R_2, Ra, R_b, R_c and R_d are all hydrogen atoms, then R_1 is not hydrogen atom or an acetyl, t-BOC, methylsulfonyl, i-propyl, methyl, ethyl, benzoyl or benzyl group;
- when m is 1 and n is 0, R is hydroxy and R_2, R_a, R_b, R_c and R_d are all hydrogen atoms, then R_1 is not hydrogen atom or t-BOC, acetoxy, or benzyl group;
- when m is 1 and n is 0, R is methyl and R_2, R_a, R_b, R_c and R_d are hydrogen atoms or methyl group, then R_1 is not hydrogen atom;
- when m is 1 and n is 0, R is ethyl or propyl group, R_a, R_b, R_c and R_d are all hydrogen atoms, then R_1 is not p-methoxyphenyl, cyclopentyl,, dichlorophenyl, cyclobutyl, cyclohexyl, p-fluorophenyl or pyridyl group;

or a pharmaceutically acceptable salt thereof.

The pyrazole-tetrahydro pyridine derivatives of formula (I), object of the invention, are obtainable through a synthetic process comprising well known reactions carried out according to conventional techniques, as well as through an extremely versatile solid-phase and/or combinatorial process, being all comprised within the scope of the invention.

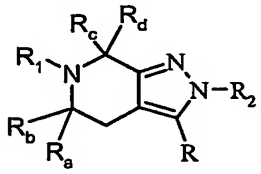
The present invention also provides a pharmaceutical composition comprising the pyrazole-tetrahydro pyridine derivatives of formula (I) and at least one pharmaceutically acceptable excipient, carrier or diluent.

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description.

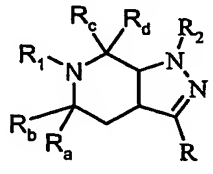
DETAILED DESCRIPTION OF THE INVENTION

The compounds of formula (I), object of the present invention, may have asymmetric carbon atoms and may therefore exist either as racemic admixtures or as individual optical isomers. Accordingly, all the possible isomers and their admixtures and of both the metabolites and the pharmaceutically acceptable bio-precursors (otherwise referred to as pro-drugs) of the compounds of formula (I), as well as any therapeutic method of treatment comprising them, are also within the scope of the present invention.

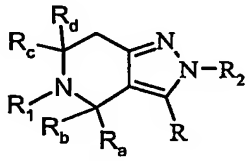
As it will be readily appreciated, the ring condensed to the pyrazole is consisting of 6 atom, and, depending on the values of n and m, two different position of the condensation are possible. As to the pyrazole ring, two isomers are possible and therefore the hydrogen atom may be on one of the two nitrogens. Accordingly, in the present invention and unless otherwise indicated, the general formula I comprises the compounds of formula IA, IB, IC, and ID:



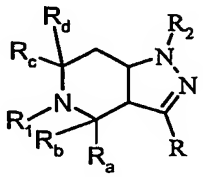
IA



IB



IC



ID

wherein R, R_1, R_2, R_a, R_b, R_c and R_d are as defined above.

As used herein, unless otherwise specified, with the term straight or branched C_1-C_6 alkyl , we intend a group such as, for instance, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, neopentyl, n-hexyl, isohexyl, and the like. With the term aryl we intend an aromatic carbocycle such as, for instance, phenyl, biphenyl, 1-naphthyl, 2-naphthyl, and the like. Clearly, aryl groups may also refer to

aromatic carbocyclic further fused or linked to non aromatic heterocyclic rings, typically 5 to 7 membered heterocycles.

With the term heterocyclyl, hence encompassing aromatic heterocycles, we further intend a saturated or partially unsaturated 5 to 7 membered carbocycle wherein one or more carbon atoms are replaced by heteroatoms such as nitrogen, oxygen and sulphur, for instance, 1,3-dioxolane, pyran, thiophene, furan, pyrrole, imidazole, pyrazole, thiazole, isothiazole, oxazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, pyrrolidine, pyrroline, imidazolidine, imidazoline, piperidine, piperazine, morpholine, tetrahydrofuran, tetrahydropyran, tetrahydrothiopyran, imidazolidine, pyrazolidine, pyrazoline, piperidine, azabicyclononane and the like.

Also the heterocycles may be optionally fused and, unless otherwise indicated, we intend any of the above defined heterocycles further condensed, through any one of the available bonds, with 5- or 6-membered, saturated or unsaturated heterocyclyl ring, or to a C_3 –C_6 cycloalkyl ring, or to a benzene or naphthalene ring such as, for instance, quinoline, isoquinoline, chroman, chromene, thionaphthene, indoline, and the like.

With the term C_2-C_6 alkenyl, we intend a straight or branched alkenyl group such as vinyl, allyl, crotyl, 2-methyl-1-propenyl, 1-methyl-1-propenyl, butenyl, pentenyl. The C_2-C_6 alkynyl group is a straight or branched alkynyl group such as ethynyl, propargyl, 1-propynyl, 1-butynyl, 2-butynyl.

With the term saturated or unsaturated C_3-C_6 cycloalkyl group we intend, for instance, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclopentenyl, cyclohexenyl, and the like. Unless otherwise specified, saturated or unsaturated cycloalkyl groups can be further condensed with 1 or 2 benzene rings are, for instance, 1,2,3,4-tetrahydro-naphthalene-2-yl, fluorene-9-yl, and the like.

The term "C_5-C_8 (hetero)cycloalkyl" as used herein refers to a 5- to 8-membered, substituted or unsubstituted, saturated or unsaturated heterocyclyl ring, containing at least one boro and two oxygen atoms, any ring carbon may be oxidized as a carbonyl, and wherein said ring may be optionally fused to a second 5- or 6-membered, saturated or unsaturated heterocyclyl ring, or to a C_3 -C_7 cycloalkyl ring, or to a benzene or naphthalene ring.

The term "aryl C_1-C_6 alkyl" refer to a straight or branched chain alkyl moiety having from 1 to 6 carbon atoms substituted with at least one aryl group as defined above, such as, for instance, benzyl, phenylethyl, benzhydryl, benzyloxy and the like. The "aryl C_2-C_6 alkenyl group" is an alkenyl group of 2 to 6 carbon atoms linked to a monocyclic or bicyclic aromatic hydrocarbon group of 6 to 10 carbon atoms. Examples of aryl alkenyl groups are styryl, 2-phenyl-1-propenyl, 3-phenyl-2-butenyl, 2-naphthylethenyl. The "aryl C_2-C_6 alkynyl group" is an alkynyl group of 2 to 6 carbon atoms linked to a monocyclic or bicyclic aromatic hydrocarbon group of 6 to 10 carbon atoms. Examples of aryl alkynyl groups are 2-phenylethynyl, 2-naphthylethynyl.

The (heterocyclyl) C_1-C_6 alkyl group is an alkyl group of 1 to 6 carbon atoms linked to a heterocyclyl group. The (heterocyclyl) C_2-C_6 alkenyl group is an alkenyl group of 2 to 6 carbon atoms linked to a heterocyclic group. The (heterocyclyl) C_2-C_6 alkynyl group is an alkynyl group of 2 to 6 carbon atoms linked to a heterocyclic group.

From all of the above, it is clear to the skilled man that any of the groups or substituents being defined, for instance, as arylalkyl, alkoxy, cycloalkoxy, aryloxy, arylalkyloxy and the like, have to be construed from the names of the groups from which they originate. As an example, unless specifically noted otherwise, any arylalkyloxy group has to be intended as an alkyloxy wherein the alkyl moiety is substituted by at least one aryl, both aryl and alkyl being as above defined.

With the term halogen atom, we intend fluoro, bromo, chloro or iodo atom.

The term "optionally substituted " means that the group may be substituted or unsubstituted; the substituents which may be present in the alkyl, cycloalkyl, aryl, arylalkyl, arylalkenyl, arylalkynyl, alkoxy, aryloxy, cycloalkoxy, alkenyl, alkynyl or heterocyclyl groups in any of the above definitions include the following:

- halo (i.e., fluoro, bromo, chloro or iodo);
- hydroxy;
- nitro;
- azido;
- mercapto (i.e., -SH), and acetyl or phenylacetyl esters thereof (i.e., $-SCOCH_3$ and $-SCOCH_2C_6H_5$);

- amino (i.e., $-NH_2$ or $-NHR^I$ or $-NR^IR^{II}$, wherein R^I and R^{II}, which are the same or different, are straight or branched C_1-C_6 alkyl, phenyl, biphenyl (i.e., $-C_6H_4-C_6H_5$), or benzyl groups, optionally substituted by hydroxy, methoxy, methyl, amino, methylamino, dimethylamino, chloro or fluoro; or R^I and R^{II} taken together with the nitrogen atom to which they are attached form a heterocyclic ring such as morpholino, pyrrolidino, piperidino, pyperazino or N-methylpyperazino;
- guanidino, i.e., $-NHC(=NH)NH_2$;
- formyl (i.e. -CHO);
- cyano;
- carboxy (i.e. -COOH), or esters thereof (i.e., $-COOR^I$), or amides thereof (i.e., $-CONH_2$, $-CONHR^I$ or $-CONHR^IR^{II}$), wherein R^I and R^{II} are as defined above, and including morpholino-amides, pyrrolidino-amides, and carboxymethylamides - $CONHCH_2COOH$;
- sulfo (i.e., $-SO_3H$);
- acyl, i.e., $-C(O)R^I$, wherein R^I is as defined above, including monofluoroacetyl, difluoroacetyl, trifluoroacetyl;
- carbamoyloxy (i.e., $-OCONH_2$) and N-methylcarbamoyloxy;
- acyloxy, i.e., $-OC(O)R^I$ wherein R^I is as defined above, or formyloxy;
- acylamino, i.e., $-NHC(O)R^I$, or $-NHC(O)OR^I$, wherein R^I is as defined above or is a group $-(CH_2)_tCOOH$ where t is 1, 2 or 3;
- ureido, i.e., $-NH(CO)NH_2$, $-NH(CO)NHR^I$, $-NH(CO)NR^IR^{II}$, wherein R^I and R^{II} are as defined above, including -NH(CO)-(4-morpholino), -NH(CO)-(1-pyrrolidino), -NH(CO)-(1-piperazino), -NH(CO)-(4-methyl-1-piperazino);
- sulfonamido, i.e., $-NHSO_2R^I$ wherein R^I is as defined above;
- a group $-(CH_2)_tCOOH$, and esters and amides thereof, i.e., $-(CH_2)_tCOOR^I$ and $-(CH_2)_tCONH_2$, $-(CH_2)_tCONHR^I$, $-(CH_2)_tCONR^IR^{II}$, wherein t, R^I and R^{II} are as defined above;
- a group $-NH(SO_2)NH_2$, $-NH(SO_2)NHR^I$, $-NH(SO_2)NR^IR^{II}$, wherein R^I and R^{II} are as defined above, including $-NH(SO_2)$-(4-morpholino), $-NH(SO_2)$-(1-pyrrolidino), $-NH(SO_2)$-(1-piperazino), $-NH(SO_2)$-(4-methyl-1-piperazino);
- a group $-OC(O)OR^I$, wherein R^I is as defined above;

- a group -OR^I, wherein R^I is as defined above, including -OCH_2COOH;
- a group –O-CH_2-O-, methylendioxy or –O-CH_2- CH_2-O-, ethylendioxy;
- a group -SR^I, wherein R^I is as defined above, including -SCH_2COOH;
- a group -$S(O)R^I$, wherein R^I is as defined above;
- a group -$S(O_2)R^I$, wherein R^I is as defined above;
- a group -SO_2NH_2, -SO_2NHR^I, or - $SO_2NR^IR^{II}$, wherein R^I and R^{II} are as defined above;
- C_1 -C_6 alkyl or C_2 -C_6 alkenyl;
- C_3 -C_7 cycloalkyl;
- substituted methyl selected from chloromethyl, fluoromethyl, difluoromethyl, trifluoromethyl, aminomethyl, N,N-dimethylaminomethyl, azidomethyl, cyanomethyl, carboxymethyl, sulfomethyl, carbamoylmethyl, carbamoyloxymethyl, hydroxymethyl, methoxycarbonylmethyl, ethoxycarbonylmethyl, tert-butoxycarbonylmethyl and guanidinomethyl.

When present, carboxy, hydroxy, mercapto and amino groups may be either free or in a protected form. Protected forms of said groups are any of those generally known in the art. Preferably, carboxy groups are protected as esters thereof, in particular methyl, ethyl, tert-butyl, benzyl, and 4-nitrobenzyl esters. Preferably, hydroxy groups are protected as silyl-ethers, ethers or esters thereof, in particular trimethyl silyl, tert-butyldiphenyl silyl, triethyl silyl, triisopropyl silyl or tert-butyldimethylsilyl ethers, methoxymethyl ethers, tetrahydropyranyl ethers, benzyl ethers, acetates or benzoates. Preferably, mercapto groups are protected as thioethers or thioesters, in particular tert-butyl thioethers, thioacetates or thiobenzoates. Preferably, amino groups are protected as carbamates, e.g. tert-butoxycarbonyl derivatives, or as amides, e.g. acetamides and benzamides.

Furthermore, hydrates, solvates of compounds of formula (I), and physiologically hydrolyzable derivatives (i.e., prodrugs) of compounds of formula (I) are included within the scope of the present invention.

With the term oxo we intend a carbonyl (>C=O) group.

With the term perfluorinated alkyl we intend any alkyl group as above defined being substituted by two or more fluorine atoms such as, for instance, trifluoromethyl, 2,2,2-trifluoroethyl, 1,1-difluoroethyl, and the like.

Pharmaceutically acceptable salts of the compounds of formula (I) are the acid addition salts with inorganic or organic, e.g. nitric, hydrochloric, hydrobromic, sulphuric, perchloric, phosphoric, acetic, trifluoroacetic, propionic, glycolic, lactic, oxalic, malonic, malic, maleic, tartaric, citric, benzoic, cinnamic, mandelic, methanesulphonic, isethionic and salicylic acid, as well as the salts with inorganic or organic bases, e.g. alkali or alkaline-earth metals, especially sodium, potassium, calcium or magnesium hydroxides, carbonates or bicarbonates, acyclic or cyclic amines, preferably methylamine, ethylamine, diethylamine, triethylamine or piperidine.

Preferred compounds of formula (I) are the compounds wherein R is H, I, Br, Cl, F, aryl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -B(OR''')$_2$, -COR' , -CONR'R", -CN, SO_2R', OR', SR', and R_1 is H, C_1-C_6 alkyl, aryl, -COR', -CONR'R", -COOR', -SO_2R', or -SO_2NR'R", and R_2 is H, -COOR', -COR', -CONR'R", C_1-C_6 alkyl, -SO_2R', or -SO_2NR'R", (heterocyclyl) C_1-C_6 alkyl group , wherein R' and R", the same or different, are selected from hydrogen or optionally substituted straight or branched C_1-C_6 alkyl, aryl or aryl C_1-C_6 alkyl groups;

R_a ,R_b, R_c and R_d, the same or different, are selected from hydrogen or straight or branched C_1-C_3 alkyl or, taken together with the carbon atom to which they are bonded form a C_3-C_6 cycloalkyl group.

Other preferred compounds of formula (I) are the compounds wherein R is selected from aryl, -COR', -CONR'R", wherein R' and R", the same or different, are selected from hydrogen or optionally substituted straight or branched C_1-C_6 alkyl, aryl or aryl C_1-C_6 alkyl groups.

Other preferred compounds of formula (I) are the compounds wherein R_1 is selected from H, C_1-C_6 alkyl, aryl, -COR', -CONR'R", COOR', -SO_2R' or -SO_2NR'R", wherein R' and R", the same or different, are selected from hydrogen or optionally substituted straight or branched C_1-C_6 alkyl, aryl or aryl C_1-C_6 alkyl groups.

Another preferred class of compounds of formula (I) are the compounds wherein R_2 is H,

-COOR', -CONR'R", C_1-C_6 alkyl, wherein R' and R", the same or different, are selected from hydrogen or optionally substituted straight or branched C_1-C_6 alkyl, aryl or aryl C_1-C_6 alkyl groups.

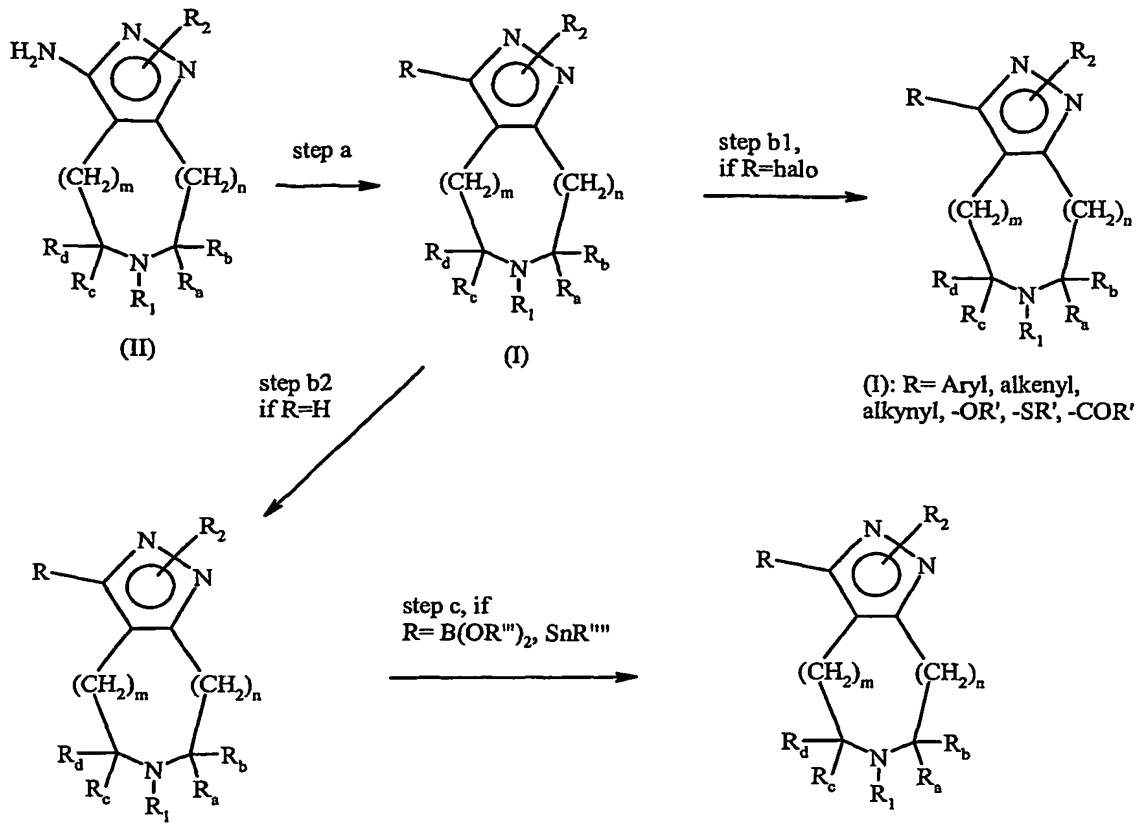
Specific, not limiting, preferred compounds of formula (I) of the invention, whenever appropriate in the form of pharmaceutically acceptable salts, are the following:

1. 5-tert-butyloxycarbonyl-1-ethoxycarbonyl-pyrazolo[4,3-c]4,5,6,7-tetrahydro pyridine;
2. 5-tert-butyloxycarbonyl-2-ethyloxycarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
3. 5-tert-butyloxycarbonyl-1(2)H- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine
4. 3-iodo-5-isopropylaminocarbonyl-1(2)H- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
5. 5-tert-butyloxycarbonyl-1-(2-trimethylsilanyl-ethyloxymethyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
6. 5-tert-butyloxycarbonyl-2-(2-trimethylsilanyl-ethyloxymethyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
7. 3-boronic acid-5-tert-butyloxycarbonyl-1-(2-trimethylsilanyl-ethoxymethyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
8. 3-boronic acid-5-tert-butyloxycarbonyl-2-(2-trimethylsilanyl-ethoxymethyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
9. 5-tert-butyloxycarbonyl-3-phenyl-1-(2-trimethylsilanyl-ethoxymethyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
10. 1-ethoxycarbonyl-5-(3-methylbutanoyl)-3-iodo- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
11. 1-ethoxycarbonyl-5-isopropylaminocarbonyl-3-iodo- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
12. 5-isopropylaminocarbonyl-3-(pyrrol-2-yl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
13. 5-tert-butyloxycarbonyl-3-(1-tert-butyloxycarbonyl-pyrrol-2-yl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
14. 5-tert-butyloxycarbonyl-3-(1-tert-butyloxycarbonyl-indol-2-yl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
15. 3-(1-tert-butyloxycarbonyl-indol-2-yl)-5-(3-methylbutanoyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

16. 5-(3-methylbutanoyl)-3-(indol-2-yl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
17. 5-tert-butyloxycarbonyl-1-ethoxycarbonyl-3-iodo- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
18. 5-tert-butyloxycarbonyl-3-iodo- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
19. 5-tert-butyloxycarbonyl-3-phenyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine:
20. 3-phenyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
21. 5-acetyl-3-phenyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
22. 5-isopropylaminocarbonyl-3-phenyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
23. 5-acetyl-3-(4-phenoxy-phenyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
24. 5-isopropylaminocarbonyl-3-(4-phenoxy-phenyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
25. 5-acetyl-3-(4-benzyloxy-phenyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
26. 3-(4-benzyloxy-phenyl)-5-isopropylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
27. 5-acetyl-3-(5-chloro-thiophen-2-yl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
28. 3-(5-chloro-thiophen-2-yl)-5-isopropylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
29. 5-acetyl-3-(4-methoxy-phenyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
30. 3-(4-methoxy-phenyl)-5-isopropylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
31. 5-acetyl-3-(4-dimethylamino-phenyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
32. 3-(4-dimethylamino-phenyl)-5-isopropylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;
33. 5-acetyl-3-phenylethynyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine and
34. 5-isopropylaminocarbonyl-3-phenylethynyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine.

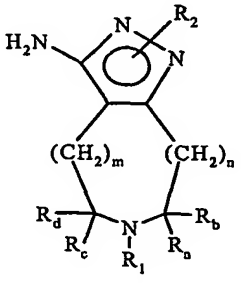
As formerly indicated, it is a further object of the invention a process for preparing the compounds of formula (I) and pharmaceutically acceptable salts thereof.

General reaction scheme



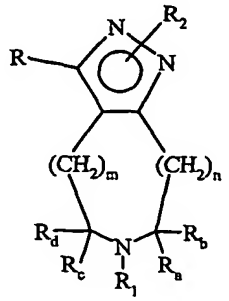
In particular, the present invention provides a process which comprises:

a) submitting a compound of formula (II)



(II)

wherein R_1 is as defined above but not hydrogen, and R_a, R_b, R_c, R_d, R_2, m and n are as defined above, to diazotation and subsequent appropriate quenching, thus obtaining a compound of formula (I)



(I)

wherein R_1 is as defined above but not hydrogen; R_a, R_b, R_c, R_d, R_2, m and n are as defined above, and R is hydrogen, iodine, bromine, chlorine or fluorine atom or a CN group;

b1) converting a thus obtained compound of formula (I) wherein R is I, Br, Cl into another compound of formula (I) wherein R is an optionally substituted aryl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -SR', -OR' or -COR' wherein R' is as defined above;

b2) converting a compound of formula (I) wherein R is hydrogen into another compound of formula (I) wherein R is -B(OR'")$_2$, -SnR"", -COOR', -COR', C_1-C_6 alkyl or iodine, wherein R', R'" and R"" are as defined above;

c) converting a compound of formula (I) wherein R is -B(OR'")$_2$ or -SnR"" as above defined into another compound of formula (I) wherein R is an optionally substituted aryl, C_2-C_6 alkenyl, C_2-C_6 alkynyl;

d) optionally converting a compound of formula (I) into another different compound of formula (I),

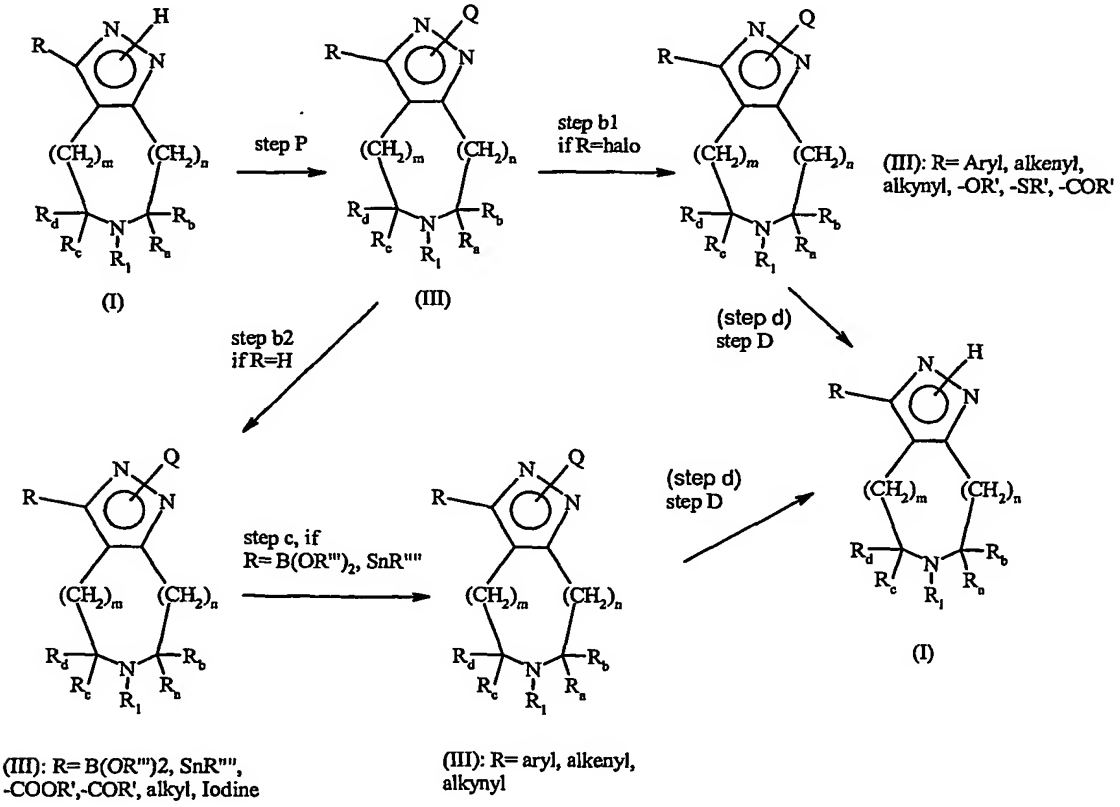
and, if desired, converting a compound of formula (I) into a pharmaceutically acceptable salt thereof or converting a salt into the free compound (I).

The above process is an analogy process which can be carried out according to well known methods. It is clear to the person skilled in the art that if a compound of formula (I), prepared according to the above process, is obtained as an admixture of isomers, their separation into the single isomers of formula (I), carried out according to conventional techniques, is still within the scope of the present invention.

Likewise, the salification of a compound of formula (I) or the conversion of its salt into the free compound (I), carried out according to well-known procedures in the art, are still within the scope of the invention.

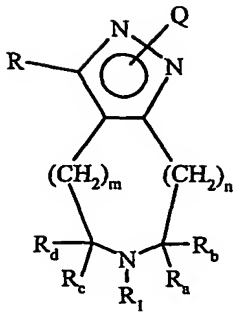
According to a preferred aspect of the process of the invention avoiding the unwanted by-products formation, a compound of formula (I), obtained according to step a above, could be first supported onto a suitable solid support, such as resin and then, after the reactions as per steps b1, b2, c and d above described, reconverted into a compound of formula (I).

General scheme



It is therefore a further object of the invention a process for preparing a compound of formula (I) as defined above, which process comprises:

P) reacting a compound of formula (I) wherein R, R_a, R_b, R_c, R_d, m and n are as defined above and R_1 is as described above but not hydrogen and R_2 is hydrogen, with a suitable solid support so as to obtain a compound of formula (III)



(III)

wherein R, R_a, R_b, R_c, R_d, m and n are as defined above, R_1 is as described above but not hydrogen, and Q is a solid support,

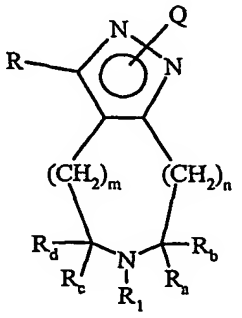
B) then, analogously to steps b1, b2, c and d above described, converting a thus obtained compound of formula (III) into another compound of formula (III) wherein R has the above reported meanings for steps b1 to d and R_1, R_a, R_b, R_c, R_d, m and n are as defined above;

D) cleaving a compound of formula (III) so as to eliminate the solid support and to obtain the desired compound of formula (I);

E) optionally converting a compound of formula (I) into another different compound of formula (I),

and, if desired, converting a compound of formula (I) into a pharmaceutically acceptable salt thereof or converting a salt into the free compound (I) as described above.

It is a further object of the present invention to provide useful intermediates of formula III



(III)

wherein R, R_1 R_a, R_b, R_c, R_d, m and n are as defined above, and Q is a solid support, more preferably a residue derived from a resin selected from the group consisting of isocyanate polystyrenic resin, 2-chloro-trityl chloride resin, trityl chloride resin, p-nitrophenyl carbonate Wang resin and the bromo-4-methoxyphenyl)methyl polystyrene.

Preferrred compounds of formula III are the following ones:

5-tert-butyloxycarbonyl-3-iodo-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-tert-butyloxycarbonyl-3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-acetyl-3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-isopropylaminocarbonyl-3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-tert-butyloxycarbonyl-3-(4-phenoxy-phenyl)- 1-polystyrenemethylaminocarbonyl-pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

3-(4-phenoxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-acetyl-3-(4-phenoxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-isopropylaminocarbonyl-3-(4-phenoxy-phenyl)-1-polystyrenemethylaminocarbonyl-pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

3-(4-benzyloxy-phenyl)-5-tert-butyloxycarbonyl-1-polystyrenemethylaminocarbonyl-pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

3-(4-benzyloxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-acetyl-3-(4-benzyloxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

3-(4-benzyloxy-phenyl)-5-isopropylaminocarbonyl-1-polystyrenemethylaminocarbonyl-pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-tert-butyloxycarbonyl-3-(5-chloro-thiphen-2-yl)- 1-polystyrenemethylaminocarbonyl-pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

3-(5-chloro-thiphen-2-yl) -1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-acetyl-3-(5-chloro-thiphen-2-yl) -1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

3-(5-chloro-thiphen-2-yl) -5-isopropylaminocarbonyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-tert-butyloxycarbonyl-3-(4-methoxy-phenyl)- 1-polystyrenemethylaminocarbonyl-pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

3-(4-methoxy-phenyl)- 1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-acetyl-3-(4-methoxy-phenyl)- 1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-isopropylaminocarbonyl-3-(4-methoxy-phenyl)- 1-polystyrenemethylaminocarbonyl-pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-tert-butyloxycarbonyl-3-(4-dimethylamino-phenyl)- 1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

3-(4-dimethylamino-phenyl)- 1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-acetyl-3-(4-dimethylamino-phenyl)- 1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

3-(4-dimethylamino-phenyl)-5-isopropylaminocarbonyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-tert-butyloxycarbonyl-3-phenylethynyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

3-phenylethynyl-1-polystyrenemethylaminocarbonyl-- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine;

5-acetyl-3-phenylethynyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine and

5-isopropylaminocarbonyl-3-phenylethynyl-1-polystyrenemethylaminocarbonyl-pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine.

A process for the preparation of a compound of formula (III) as defined above is also provided, which process comprises:

P) reacting a compound of formula (I) wherein R, R_a, R_b, R_c, R_d, m and n are as defined above, R_1 is as described above but not hydrogen and R_2 is hydrogen, with a suitable solid support so as to obtain a compound of formula (III) wherein R, R_a, R_b, R_c, R_d, m and n are as defined above, R_1 is as described above but not hydrogen, and Q is a solid support,

B) then, analogously to steps b1, b2, c and d above described, optionally converting a thus obtained compound of formula (III) into another compound of formula (III) wherein R has the above reported meanings for steps b1 to d and R_1, R_a, R_b, R_c, R_d, m and n are as defined above.

According to step a) of the process, a compound of formula (I) wherein R is hydrogen, I, Br, Cl, F, CN, and R_1 is as defined above but not hydrogen, and R_a, R_b, R_c, R_d, R_2, m and n are as defined above, may be prepared by reacting a compound of formula (II), wherein R_1 is as defined above but not hydrogen, and R_a, R_b, R_c, R_d, R_2, m and n are as defined above, with organic or inorganic nitrites such as sodium nitrite or isopentylnitrite, in the presence of a suitable hydrogen source, such as H_3PO_2, HMPT (hexamethylphosphorus triamide), thiophenol, sodium stannite, Bu_3SnH, Et_3SiH, or of a suitable halogenating or cyanating agent such as tetrabutylamonium iodide and/or iodine, tetrabutylamonium bromide and/or bromine, tetrabutylamonium chloride and/or chlorine, CuBr, CuCl, CuI, CuCN, sodium tetrafluoroborate, ammonium tetrafluoroborate, in aqueos acidic solution at various concentrations such as diluted chloridic acid or diluted citric acid, or in organic solvents such as tetrahydrofurane, 1,4-dioxane, dichloromethane, chloroform, toluene, acetonitrile, ethylacetate, acetone, dimethylformamide, ethanol, methanol, water at a temperature ranging from about -78° C to reflux, for a suitable time ranging from 5 min to 72 hours. More preferably, the step a) is carried out on compounds of the formula (II) wherein R_2 is not hydrogen atom.

According to step b1) of the process, a compound of formula (I) wherein R is an optionally substituted aryl or C_2-C_6 alkenyl group, and R_1, R_2, R_a, R_b, R_c, R_d, m and n are as defined above, can be obtained by reacting a compound of formula (I), wherein R is halogen atom, and R_1, R_2, R_a, R_b, R_c, R_d, m and n are as defined above, with a suitable aryl boronic acid or ester, alkenyl boronic acid or ester, arylstannane, in the presence of a suitable catalysing agent such as palladium(0)tetrakis, bis triphenylphosphine palladium(II) dichloride, bis tricyclohexylphosphine palladium(II) dichloride, bis tri-o-tolylphosphine palladium(II) dichloride, palladium(II) acetate, tris(dibenzylideneacetone) dipalladium(0), [1,1'-bis(diphenylphosphino) ferrocene] dichloropalladium(II), [1,1'-bis(diphenylphosphino) ferrocene] dichloronickel(II), 1,4-bis(diphenylphosphino) butane palladium(II), and of a suitable base such as sodium carbonate, cesium carbonate, potassium carbonate, potassium phosphate, triethylamine, sodium hydroxide, cesium fluoride, potassium tert-butylate, sodium ethylate, potassium acetate, in a suitable solvent, such as 1,4-dioxane, tetrahydrofurane, DMF (N,N-dimethylformamide), dimethoxyethane, toluene, methanol, ethanol, water, N-methylpyrrolidone, and, when needed, adding a suitable ligand, such as tributylphosphine, triphenylphosphine, tri-o-tolylphosphine, tricyclohexyl, biphenyl(dicyclohexyl) phosphine, biphenyl(ditert-butyl) phosphine, diphenylphosphine ferrocene , and/or Cu(I) salts such as CuI, Cu(I)thiphene-2-carboxylate at a temperature ranging from room temperature to reflux, for a suitable time ranging from 15 minutes to 72 hours.

According to step b1) of the process, a compound of formula (I) wherein R is an optionally substituted C_1-C_6 alkynyl, and R_1, R_2, R_a, R_b, R_c, R_d, m and n are as defined above, can be obtained by reacting a compound of formula (I), wherein R is halogen, and R_1, R_2, R_a, R_b, R_c, R_d, m and n are as defined above, with a suitable alkyne under the condition of the Sonogashira's reaction, in the presence of a suitable catalysing agent such as bistriphenylphosine palladium(II) dichloride, palladium(0) tetrakis, palladium(II) acetate, tris(dibenzylideneacetone) dipalladium(0), and of a suitable Cu(I) salt , such as CuI, and in presence of a suitable base such as sodium carbonate, potassium carbonate, cesium carbonate, potassium phosphate, triethylamine, diisopropylamine, pyridine, in a suitable solvent, such as 1,4-dioxane,

tetrahydrofurane, DMF, dimethoxyethane, toluene, ethanol, methanol, and, if needed, adding a suitable ligand such as triphenylphosphine, tri-o-tolylphosphine, tricyclohexyl, diphenylphosphineferrocene, at a temperature ranging from room temperature to reflux, for a suitable time ranging from 15 minutes to 72 hours.

According to step b1) of the process, a compound of formula (I) wherein R is SR', OR', and R_1, R_2, R_a, R_b, R_c, R_d, R', m and n are as defined above, can be obtained by reacting a compound of formula (I), wherein R is halogen, and R_1, R_2, R_a, R_b, R_c, R_d, m and n are as defined above, with a suitable alcohols or thiols R'OH or R'SH wherein R' is as above defined, in the presence of a suitable base, such as, potassium carbonate, sodium carbonate, cesium carbonate,potassium hydroxide, sodium hydroxide, sodium hydride, sodium methylate, sodium tert-butylate, diisopropylethylamine, pyridine, piperidine, N-methylmorpholine, dimethylaminopyridine, and, if needed, in the presence of catalysing agent, such as bis tricyclohexylphosphine palladium(II) dichloride, bis tri-o-tolylphosphine palladium(II) dichloride, palladium(II) acetate, tris(dibenzylideneacetone) dipalladium(0), [1,1'-bis(diphenylphosphino) ferrocene] dichloropalladium(II), and of a suitable ligand, such as, triphenylphosphine, tri-o-tolylphosphine, tricyclohexyl, diphenylphosphineferrocene, in a suitable solvent, such as dimethylformamide, NMP,dichloromethane, tetrahydrofurane, benzene, toluene, pyridine, dimethylsulfoxide at a temperature ranging from - 20°C to reflux, for a suitable time ranging from 15 minutes to 72 hours.

According to step b1) of the process, a compound of formula (I) wherein R is -COR', and R_1, R_2, R_a, R_b, R_c, R_d, m and n are as defined above, can be obtained by reacting a compound of formula (I) wherein R is halogen and R_1, R_2, R_a, R_b, R_c, R_d, m and n are as defined above, with a suitable base, such as n-butyl lithium, LDA (lithium diisopropylamide, sec-butyl lithium, t-butyl lithium, lithium 2,2,6,6-tetramethylpiperidin amide, phenyl lithium, magnesium, isopropylmagnesium bromide in a suitable solvent, such as diethyl ether, tetrahydrofurane, 1,4-dioxane, n-hexane, cyclohexane, pentane, toluene, DME (ethylene glycol dimethyl ether), dimethylsulfoxide in the presence of a base if needed, such as TMEDA (N,N,N',N'-tetramethylethylenediamine), at a suitable temperature ranging from –78°C to room temperature, for a time ranging from 15 minutes to 3 hours; the resulting lithium

derivative can be quenched with a suitable electrophylic agent, such as, trialkylarylstannane/carbon monoxide, acid chlorides, acid fluorides, acid bromides, anhydrides, carbonates, halo carbonates, carbamates, DMF, and if needed, in the presence of a suitable catalysing agent, such as Pd(0)tetrakis, and of a suitable cohordinating agent, such as $ZnCl_2$, $ZnBr_2$, CuCN.2LiCl, CuI, CuBr, $CuBr.SMe_2$ at a suitable temperature ranging from about –78°C to reflux, for a time ranging from 15 minutes to about 72 hours.

According to step b2) of the process, a compound of formula (I) wherein R is iodine, $B(OR''')_2$, SnR'''', -COOR', -COR', C_1-C_6 alkyl and R_1, R_2, R_a, R_b, R_c, R_d, R', R''', R'''', m and n are as defined above, can be obtained by reacting a compound of formula (I) wherein R is hydrogen and R_1, R_2, R_a, R_b, R_c, R_d, m and n are as defined above, with a suitable lithiating agent, such as n-butyl lithium, LDA, sec-butyl lithium, t-butyl lithium, lithium 2,2,6,6-tetramethylpiperidinamide, phenyl lithium, in a suitable solvent, such as diethyl ether, tetrahydrofurane, 1,4-dioxane, n-hexane, cyclohexane, toluene, DME, dimethylsulfoxide in the presence of a base if needed, such as TMEDA, at a suitable temperature ranging from –78°C to room temperature, for a time ranging from 15 minutes to 3 hours; the resulting lithium derivative can be quenched with a suitable electrophylic agent, such as trialkyl boronic esters, trialkylstannyl chloride, acid chlorides, acid fluorides, acid bromides, anhydrides, carbonates, halo carbonates, DMF, iodine, aldehydes, ketones, alkyl halides, in the presence of a suitable cohordinating agent, such as $ZnCl_2$, $ZnBr_2$, CuCN.2LiCl, CuI, CuBr, $CuBr.SMe_2$ when needed, at a suitable temperature ranging from about –78°C to reflux, for a time ranging from 15 minutes to about 72 hours.

According to step c) of the process, a compound of formula (I) wherein R is an optionally substituted aryl or C_1-C_6 alkenyl group and R_1, R_2, R_a, R_b, R_c, R_d, m and n are as defined above, can be obtained by reacting a compound of formula (I) wherein R is $B(OR''')_2$, SnR'''', and R_1, R_2, R_a, R_b, R_c, R_d, R''', R'''', m and n are as defined above, with a suitable aryl halide or halogeno olefine, in the presence of a suitable catalysing agent such as as palladium(0)tetrakis, bis triphenylphosphine palladium(II) dichloride, bis tricyclohexylphosphine palladium(II) dichloride, bis tri-o-tolylphosphine palladium(II) dichloride, palladium(II) acetate, tris(dibenzylideneacetone)

dipalladium(0), [1,1'-bis(diphenylphosphino) ferrocene] dichloropalladium(II), [1,1'-bis(diphenylphosphino) ferrocene] dichloronickel(II), 1,4-bis(diphenylphosphino) butane palladium(II), as sodium carbonate, cesium carbonate, potassium carbonate, potassium phosphate, triethylamine, sodium hydroxide, cesium fluoride, potassium tert-butylate, sodium ethylate, potassium acetate,, in a suitable solvent, such as 1,4-dioxane, tetrahydrofurane, DMF, dimethoxyethane, toluene, methanol, ethanol, water, N-methylpyrrolidone and, if needed, adding a suitable ligand, such as tributylphosphine, triphenylphosphine, tri-o-tolylphosphine, tricyclohexyl, biphenyl(dicyclohexyl)phosphine, biphenyl(ditert-butyl)phosphine, diphenylphosphineferrocene , and/or a suitable Cu(I) salts, such as CuI, Cu(I)thiophene-2-carboxylate at a temperature ranging from room temperature to reflux, for a suitable time ranging from 15 minutes to 72 hours.

According to step c) of the process, a compound of formula (I) wherein R is an optionally substituted C_2-C_6 alkynyl, and R_1, R_2, R_a, R_b, R_c, R_d, m and n are as defined above, can be obtained by reacting a compound of formula (I) wherein R is $B(OR''')_2$, SnR'''', and R_1, R_2, R_a, R_b, R_c, R_d, R''', R'''', m and n are as defined above, with a suitable 1-alkyl(aryl)thio-alkyne, 1-iodo(bromo)alkyne, or 1,1-dibromo-1-alkene, in the presence of a suitable catalysing agent such as as palladium(0)tetrakis, bis triphenylphosphine palladium(II) dichloride, bis tricyclohexylphosphine palladium(II) dichloride, bis tri-o-tolylphosphine palladium(II) dichloride, palladium(II) acetate, tris(dibenzylideneacetone) dipalladium(0), [1,1'-bis(diphenylphosphino) ferrocene] dichloropalladium(II), [1,1'-bis(diphenylphosphino) ferrocene] dichloronickel(II), 1,4-bis(diphenylphosphino) butane palladium(II) in a suitable solvent, such as 1,4-dioxane, tetrahydrofurane, DMF, dimethoxyethane, toluene, methanol, ethanol, water, N-methylpyrrolidone and, if needed, adding a suitable ligand, such as tributylphosphine, triphenylphosphine, tri-o-tolylphosphine, tricyclohexyl, biphenyl(dicyclohexyl)phosphine, biphenyl(ditert-butyl)phosphine, diphenylphosphineferrocene , and/or a suitable Cu(I) salts, such as CuI, Cu(I)thiophene-2-carboxylate at a temperature ranging from room temperature to reflux, for a suitable time ranging from 15 minutes to 72 hours.

According to step P of the process, a compound of formula (III) wherein R, R_a, R_b, R_c, R_d, m and n are as described above, R_1 is as described above but not hydrogen and Q is a solid support can be obtained by reacting a compound of formula (I) wherein R, R_a, R_b, R_c, R_d, m and n are as described above, R_1 is described above but not hydrogen and R_2 is hydrogen, with a suitable solid support such as a polymeric support like isocyanate polystyrenic resin, 2-chloro-trityl chloride resin, trityl chloride resin, p-nitrophenyl carbonate Wang resin or the bromo-4-methoxyphenyl)methyl polystyrene, which are all conventionally known in this field, in the presence, when needed, of a suitable base, such as diisopropylethylamine, triethylamine, 1,8-diazabiciclo[5.4.0] undec-7-ene or 2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro -1,3,2-diaza-phosphorine, in a suitable solvent such as dichloromethane, chloroform, tetrahydrofuran, dimethylformamide, dimethylacetamide, 1-methyl-2-pyrrolidinone, dimethylsulfoxide and the like, at a temperature ranging from room temperature to 50°C, for a suitable time ranging from 10 minutes to 90 hours.

According to step B of the process, a compound of formula (III) may be converted into a different compound of formula (III) by steps analogous to the steps b1), b2), c) and d) herein described for the conversion of a compound of the formula (I) into a different compound of formula (I).

According to step D of the process a compound of formula (I) wherein R, R_a, R_b, R_c, R_d, m and n are as described above, R_1 is as described above and R_2 is hydrogen, can be obtained by cleaving a compound (III) wherein R, R_a, R_b, R_c, R_d, m and n are as described above, R_1 is as described above and Q is a solid support, according to conventional hydrolytic methods in the presence of a suitable acid, such as hydrochloric acid, acetic acid, trifluoroacetic acid, hydrofluoric acid, or in the presence of a suitable base, such as sodium hydroxide, potassium hydroxide, sodium carbonate, sodium hydrogencarbonate, piperidine, or in the presence of other hydrolytic agents, such as tetrabutyl ammoniumfluoride, trimethyl silylchloride, in a suitable solvent such as dichloromethane, chloroform, methanol, ethanol, trifluoroethanol, dioxane, at a temperature ranging from room temperature to 70°C, for a suitable time ranging from 10 minutes to 90 hours.

According to step E of the process, a compound of formula (I) wherein R, R_a, R_b, R_c, R_d, m and n are as described above, R_1 is as described above and R_2 is hydrogen may be converted into another different compound of formula (I), the conversion being carried out in several ways, depending on the meanings of the substituents and the presence of other substituents in the molecule. For example, by this conversion a compound of formula (I)

wherein R_2 is as defined above but not hydrogen may be obtained.

According to step d) of the process, the conversion of a compound of formula (I) into another different compound of formula (I) may be carried out in several ways, depending on the meanings of the substituents and the presence of other substituents in the molecule. For example, a conversion can be a hydrolysis, a reductive amination, an arylation, an alkylation, an amination, a nucleophylic substitution, a catalytic reduction, an oxidation, a reduction, a condensation with an appropriate reagent or a combination of these reactions.

As an example, the compounds of formula (I) or (III), wherein R_1 is -COO^{t}Bu can be hydrolized to the corresponding compounds of formula (I) wherein R_1 is H, by treatment with a suitable acid, for instance trifluoroacetic or hydrochloric acid.

So far, any of the above compounds of formula (I) or (III) wherein R_1 is a hydrogen atom can be easily converted into the corresponding derivatives alkylated, acylated, sulfonated or arylated. The reactions are carried out according to conventional techniques, for istance by properly reacting the amino derivative (I) or (III) wherein R1 is hydrogen with alkylating, acylating, sulfonylating or arylating agents and the like.

In particular, a compound of formula (I) or (III) wherein R_1 is selected from R' other than hydrogen, -COR', -COOR', -CONR'R", -SO_2R', or -SO_2NR'R", wherein R' and R" have the above reported meanings; R, R_2 and R_a, R_b, R_c, R_d, m and n are as above defined, may be prepared by reacting a compound of formula (I) or a compound of formula (III), having R_1 equal to hydrogen, with a compound of formula (IV)

$$R_1\text{-}X \quad (IV)$$

wherein R_1 is as above defined but not hydrogen and X is a suitable leaving group, preferably fluorine, chlorine, bromine or iodine.

The above reaction can be carried out according to conventional procedures well known in the art for acylating, sulfonylating, alkylating or arylating amino groups, for instance in the presence of a suitable base, such as potassium carbonate, triethylamine, N,N-diisopropylethylamine or pyridine, in a suitable solvent such as dimethylsulfoxide, toluene, dichloromethane, chloroform, diethyl ether, tetrahydrofuran, acetonitrile, or N,N-dimethylformamide, at a temperature ranging from about -10°C to reflux and for a time varying from about 30 minutes to about 96 hours.

A compound of formula (I) or (III) wherein R_1 is an aryl group, R, R_2 and R_a, R_b, R_c, R_d, m and n are as above defined, may be prepared by reacting a compound of formula (I) or a compound of formula (III), having R_1 equal to hydrogen with a compund of formula (V)

$$R_1\text{-}X \quad (V)$$

wherein R_1 is an aryl group and X is as above defined. The above reaction can be carried out according to conventional procedures well known in the art for arylating amino groups, for instance in the presence of a suitable catalyst when needed, such as palladium(0)tetrakis, bistriphenylphosphinePalladium(II)chloride, bis tricyclohexylphosphine palladium(II) dichloride, bis tri-o-tolylphosphine palladium(II) dichloride, palladium(II) acetate, tris(dibenzylideneacetone) dipalladium(0), [1,1'-bis(diphenylphosphino) ferrocene] dichloropalladium(II), as sodium carbonate, cesium carbonate, potassium carbonate, potassium phosphate, triethylamine, sodium hydroxide, cesium fluoride, potassium tert-butylate, sodium tert-butylate, sodium ethylate, potassium acetate, in a suitable solvent, such as 1,4-dioxane, tetrahydrofurane, DMF, dimethilsulfoxide, dimethoxyethane, toluene, methanol, ethanol, water, N-methylpyrrolidone and adding a suitable ligand, such as tributylphosphine, triphenylphosphine, tri-o-tolylphosphine, tricyclohexyl, biphenyl(dicyclohexyl)phosphine, biphenyl(ditert-butyl)phosphine, diphenylphosphineferrocene , BINAP [(2,2'-bis(diphenylphosphino)-1,1'-binaphthyl], and adding, when needed a phase transfer catalysing agent, such as 18-crown-6, at a temperature ranging from room temperature to reflux, for a suitable time ranging from 15 minutes to 72 hours.

From the foregoing it is clear to the person skilled in the art that the preparation of the compounds of formula (I) or (III) having R_1 equal to $-SO_2NR'R''$ can be actually performed as above described or, alternatively, by properly reacting a compound of formula (I) or (III) having R_1 equal to $-SO_2NHR'$ with any suitable alkylating moiety, according to well known methodologies for preparing di-substituted sulfonamides.

A compound of formula (I) or (III) wherein R_1 is a -CONHR' group, R' has the above reported meanings other than hydrogen, R, R_2, and R_a, R_b, R_c, R_d, m and n are as above defined, may be prepared by reacting a compound of formula (I) or a compound of formula (III) having R_1 equal to hydrogen, with a compound of formula (VI)

R'-NCO (VI)

wherein R' is as above defined but not hydrogen, so as to obtain a corresponding compound of formula (I) or (III) which may be optionally further reacted with a compound of formula (VII)

R''-X (VII)

wherein R'' is as above defined other than hydrogen and X is as above defined, so as to obtain a compound of formula (I) or (III) wherein R_1 is -CONR'R'', wherein R' and R'' are as above defined but not hydrogen atom.

The reaction between the above compounds (I) or (III) with a compound of formula (VII) can be carried out in the presence of a tertiary base, such as triethylamine, N,N-diisopropylethylamine or pyridine, in a suitable solvent, such as toluene, dichloromethane, chloroform, diethyl ether, tetrahydrofuran, acetonitrile, or N,N-dimethylformamide, at a temperature ranging from about -10°C to reflux and for a time varying from about 30 minutes to about 72 hours.

The optional subsequent conversion of a compound of formula (I) or (III) having R_1 equal to -CONHR' into a corresponding derivative having R_1 equal to -CONR'R'' is carried out according to conventional methods used to prepare di-substituted ureido derivatives.

A compound of formula (I) or (III) wherein R_1 is a -CONR'R'' group, R' and R'' has the above reported meanings other than hydrogen, R, R_2 and R_a, R_b, R_c, R_d, m and n are as above defined, may be prepared by reacting a compound of formula (I) or a compound

of formula (III) having R_1 equal to hydrogen with 4-nitrophenylchloroformate and subsequently with a compound of formula (VIII)

R'R"NH (VIII)

wherein R' and R" are as defined above but not hydrogen.

The reaction is carried out according to conventional methods used to prepare di-substituted ureido derivatives.

Alternatively, a compound of formula (I) or a compound of formula (III), having R_1 equal to hydrogen may be reacted under reductive conditions with a compound of formula (IX)

R'-CHO (IX)

wherein R' is as defined above but not hydrogen, so as to obtain a corresponding compound of formula (I) or (III) wherein R_1 is a $-CH_2R'$ group and R' being as defined above but not hydrogen.

The reaction is carried out in a suitable solvent such as, for instance, N,N-dimethylformamide, N,N-dimethylacetamide, chloroform, dichloromethane, tetrahydrofuran, or acetonitrile, optionally in the presence of acetic acid, ethanol or methanol as co-solvents, at a temperature ranging from about -10°C to reflux and for a time varying from about 30 min to about 4 days.

Conventional reducing agents in the reaction medium are, for instance, sodium boron hydride, sodium triacethoxy boron hydride, and the like.

In a further example, any of the above compounds of formula (I) or of formula (III) wherein one or more of R_a, R_b, R_c and R_d is $-CH_2OH$ may be conveniently prepared by starting from a corresponding protected derivative having one or more of R_a, R_b, R_c and R_d as $-CH_2-O-Si(Me)_2tBu$ or $-CH_2-O-Ph$.

The reaction is carried according to conventional techniques, for instance in a suitable solvent such as, for instance, N,N-dimethylformamide, chloroform, dichloromethane, tetrahydrofuran, methanol, ethanol or acetonitrile, at a temperature ranging from about -10°C to reflux and for a time varying from about 30 min to about 72 hours with a suitable fluoride source, for instance tetrabutylamonium fluoride.

Likewise, the above compounds of formula (I) or (III) having one or more R_a, R_b, R_c and R_d equal to $-CH_2OH$ can be reacted with a compound of formula (VII')

R'-X (VII')

wherein R' is as above defined but not hydrogen and X is as above defined, so as to obtain the corresponding compounds wherein one or more R_a, R_b, R_c and R_d are a $-CH_2OR'$ group, wherein R' is as defined above but not hydrogen.

This latter reaction can be carried out in the presence of a base, such as sodium hydride, N,N-diisopropylethylamine or pyridine, in a suitable solvent, such as toluene, dichloromethane, chloroform, diethyl ether, tetrahydrofuran, acetonitrile, or N,N-dimethylformamide, at a temperature ranging from about -10°C to reflux.

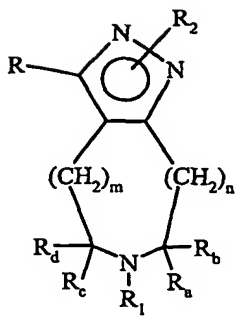
The starting compound of formula (II) are known or can be prepared starting from known compounds using known method of prparation, for example those described in WO02/12242. As it will be really appreciated by the man skilled in the art, when preparing the compounds of formula (I) object of the invention, optional functional groups within both the starting materials or the intermediates thereof which could give rise to unwanted side reactions, need to be properly protected according to conventional techniques. Likewise, the conversion of these latter into the free deprotected compounds may be carried out according to known procedures.

The above cited reagents of the process, i.e. arylboronic acids, arylboronic esters, alkenylboronic acids, alkenylboronic esters, triarylstannanes, acid chlorides, acid fluorides, acid bromides, anhydrides, carbonates, halo carbonates, alkynes, aryl halides, halogeno alkenes and the compounds of formula (IV), (V), (VI), (VII), (VII'), (VIII) and (IX) are known or can be prepared according to known methods.

As it will be also really appreciated by the man skilled in the art, when preparing the compounds of formula (I) object of the invention, according to steps a)-c), each of the above cited reactants can be replaced by the corresponding polymer-supported reactant.

In addition to the above, it is also clear to the skilled man that the compounds of formula (I) of the invention can be advantageously prepared by combining the above described reactions in a combinatorial fashion, for example according to solid-phase-synthesis (SPS) techniques, so as to get a combinatorial chemical library of compounds of formula (I).

It is therefore a further object of the invention a library of two or more compounds of formula (I):



(I)

wherein R, R_1, R_2 R_a, R_b, R_c, R_d m and n are as defined above, which can be obtained starting from one or more compound supported onto a solid support of the formula (III) as defined above.

PHARMACOLOGY

The compounds of formula (I) are active as protein kinase inhibitors and are therefore useful, for instance, to restrict the unregulated proliferation of tumor cells.

In therapy, they may be used in the treatment of various tumors, such as those formerly reported, as well as in the treatment of other cell proliferative disorders such as psoriasis, vascular smooth cell proliferation associated with atherosclerosis and post-surgical stenosis and restenosis and in the treatment of Alzheimer's disease.

The inhibiting activity of putative cdk/cyclin inhibitors and the potency of selected compounds is determined through a method of assay based on the use of the SPA technology (Amersham Pharmacia Biotech).

The assay consists of the transfer of radioactivity labelled phosphate moiety by the kinase to a biotinylated substrate. The resulting 33P-labelled biotinylated product is allowed to bind to streptavidin-coated SPA beads (biotin capacity 130 pmol/mg), and light emitted was measured in a scintillation counter.

Inhibition assay of cdk2/Cyclin A activity

Kinase reaction: 4 μM in house biotinylated histone H1 (Sigma # H-5505) substrate, 10 μM ATP (0.1 microCi $P^{33}\gamma$-ATP), 1.1 nM Cyclin A/CDK2 complex, inhibitor in a final volume of 30 μl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After incubation for 60 min at room temperature, the reaction was stopped by addition of 100 μl PBS buffer

containing 32 mM EDTA, 500 μM cold ATP, 0.1% Triton X100 and 10mg/ml streptavidin coated SPA beads. After 20 min incubation, 110 μL of suspension were withdrawn and transferred into 96-well OPTIPLATEs containing 100 μl of 5M CsCl. After 4 hours, the plates were read for 2 min in a Packard TOP-Count radioactivity reader.

IC50 determination: inhibitors were tested at different concentrations ranging from 0.0015 to 10 μM. Experimental data were analyzed by the computer program GraphPad Prizm using the four parameter logistic equation:

y = bottom+(top-bottom)/(1+10^((logIC50-x)*slope))

where x is the logarithm of the inhibitor concentration, y is the response; y starts at bottom and goes to top with a sigmoid shape.

Ki calculation:

Experimental method: Reaction was carried out in buffer (10 mM Tris, pH 7.5, 10 mM $MgCl_2$, 0.2 mg/ml BSA, 7.5 mM DTT) containing 3.7 nM enzyme, histone and ATP (constant ratio of cold/labeled ATP 1/3000). Reaction was stopped with EDTA and the substrate captured on phosphomembrane (Multiscreen 96 well plates from Millipore). After extensive washing, the multiscreen plates were read on a top counter. Control (time zero) for each ATP and histone concentrations was measured.

Experimental design: Reaction velocities are measured at four ATP, substrate (histone) and inhibitor concentrations. An 80-point concentration matrix was designed around the respective ATP and substrate Km values, and the inhibitor IC50 values (0.3, 1, 3, 9 fold the Km or IC50 values). A preliminary time course experiment in the absence of inhibitor and at the different ATP and substrate concentrations allows the selection of a single endpoint time (10 min) in the linear range of the reaction for the Ki determination experiment.

Kinetic parameter estimates: Kinetic parameters were estimated by simultaneous nonlinear least-square regression using [Eq.1] (competitive inhibitor respect to ATP, random mechanism) using the complete data set (80 points):

$$v = \frac{Vm \bullet A \bullet B}{\alpha \bullet Ka \bullet Kb + \alpha \bullet Ka \bullet B + a \bullet Kb \bullet A + A \bullet B + \alpha \bullet \frac{Ka}{Ki} \bullet I \bullet (Kb + \frac{B}{\beta})} \quad \text{[Eq.1]}$$

where A=[ATP], B=[Substrate], I=[inhibitor], Vm= maximum velocity, Ka, Kb, Ki the dissociation constants of ATP, substrate and inhibitor respectively. α and β the cooperativity factor between substrate and ATP binding and substrate and inhibitor binding respectively.

In addition the selected compounds are characterized on a panel of ser/thre kinases strictly related to cell cycle (cdk2/cyclin E, cdk1/cyclin B1, cdk5/p25, cdk4/ cyclin D1), and also for specificity on MAPK, PKA, EGFR, IGF1-R, Aurora-2 and Cdc 7

Inhibition assay of cdk2/Cyclin E activity

Kinase reaction: 10 μM in house biotinylated histone H1 (Sigma # H-5505) substrate, 30 μM ATP (0.3 microCi $P^{33}\gamma$-ATP), 4 ng GST-Cyclin E/CDK2 complex, inhibitor in a final volume of 30 μl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After incubation for 60 min at room temperature, the reaction was stopped by addition of 100 μl PBS buffer containing 32 mM EDTA, 500 μM cold ATP, 0.1% Triton X100 and 10mg/ml streptavidin coated SPA beads. After 20 min incubation, 110 μL of suspension were withdrawn and transferred into 96-well OPTIPLATEs containing 100 μl of 5M CsCl. After 4 hours, the plates were read for 2 min in a Packard TOP-Count radioactivity reader.

IC50 determination: see above

Inhibition assay of cdk1/Cyclin B1 activity

Kinase reaction: 4 μM in house biotinylated histone H1 (Sigma # H-5505) substrate, 20 μM ATP (0.2 microCi $P^{33}\gamma$-ATP), 3 ng Cyclin B/CDK1 complex, inhibitor in a final volume of 30 μl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After 20 min at r.t. incubation, reaction was stopped by 100 μl PBS + 32 mM EDTA + 0.1% Triton X-100 + 500 μM ATP, containing 1 mg SPA beads. Then a volume of 110 μl is transferred to Optiplate.

After 20 min. incubation for substrate capture, 100 µl 5M CsCl were added to allow statification of beads to the top of the Optiplate and let stand 4 hours before radioactivity counting in the Top-Count instrument.

IC50 determination: see above

Inhibition assay of cdk5/p25 activity

The inhibition assay of cdk5/p25 activity is performed according to the following protocol.

Kinase reaction: 10 µM biotinylated histone H1 (Sigma # H-5505) substrate, 30 µM ATP (0.3 microCi $P^{33}\gamma$-ATP), 15 ng CDK5/p25 complex, inhibitor in a final volume of 30 µl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After incubation for 35 min at room temperature, the reaction was stopped by addition of 100 µl PBS buffer containing 32 mM EDTA, 500 µM cold ATP, 0.1% Triton X100 and 10 mg/ml streptavidin coated SPA beads. After 20 min incubation, 110 µL of suspension were withdrawn and transferred into 96-well OPTIPLATEs containing 100 µl of 5M CsCl. After 4 hours, the plates were read for 2 min in a Packard TOP-Count radioactivity reader.

IC50 determination: see above

Inhibition assay of cdk4/Cyclin D1 activity

Kinase reaction: 0,4 uM µM mouse GST-Rb (769-921) (# sc-4112 from Santa Cruz) substrate, 10 µM ATP (0.5 µCi $P^{33}\gamma$-ATP), 100 ng of baculovirus expressed GST-cdk4/GST-Cyclin D1, suitable concentrations of inhibitor in a final volume of 50 µl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, 7.5 mM DTT+ 0.2mg/ml BSA) were added to each well of a 96 U bottom well plate. After 40 min at 37 °C incubation, reaction was stopped by 20 µl EDTA 120 mM.

Capture: 60 µl were transferred from each well to MultiScreen plate, to allow substrate binding to phosphocellulose filter. Plates were then washed 3 times with 150 µl/well PBS Ca^{++}/Mg^{++} free and filtered by MultiScreen filtration system.

Detection: filters were allowed to dry at 37°C, then 100 µl/well scintillant were added and ^{33}P labeled Rb fragment was detected by radioactivity counting in the Top-Count instrument.

IC50 determination: see above

Inhibition assay of MAPK activity

Kinase reaction: 10 μM in house biotinylated MBP (Sigma # M-1891) substrate, 15 μM ATP (0.15 microCi $P^{33}\gamma$-ATP), 30 ng GST-MAPK (Upstate Biothecnology # 14-173), inhibitor in a final volume of 30 μl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After incubation for 35 min at room temperature, the reaction was stopped by addition of 100 μl PBS buffer containing 32 mM EDTA, 500 μM cold ATP, 0.1% Triton X100 and 10mg/ml streptavidin coated SPA beads. After 20 min incubation, 110 μL of suspension were withdrawn and transferred into 96-well OPTIPLATEs containing 100 μl of 5M CsCl. After 4 hours, the plates were read for 2 min in a Packard TOP-Count radioactivity reader.

IC50 determination: see above

Inhibition assay of PKA activity

Kinase reaction: 10 μM in house biotinylated histone H1 (Sigma # H-5505) substrate, 10 μM ATP (0.2 microM $P^{33}\gamma$-ATP), 0.45 U PKA (Sigma # 2645), inhibitor in a final volume of 30 μl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After incubation for 90 min at room temperature, the reaction was stopped by addition of 100 μl PBS buffer containing 32 mM EDTA, 500 μM cold ATP, 0.1% Triton X100 and 10mg/ml streptavidin coated SPA beads. After 20 min incubation, 110 μL of suspension were withdrawn and transferred into 96-well OPTIPLATEs containing 100 μl of 5M CsCl. After 4 hours, the plates were read for 2 min in a Packard TOP-Count radioactivity reader.

IC50 determination: see above

Inhibition assay of EGFR activity

Kinase reaction: 10 μM in house biotinylated MBP (Sigma # M-1891) substrate, 2 μM ATP (0.04 microCi $P^{33}\gamma$-ATP), 36 ng insect cell expressed GST-EGFR, inhibitor in a final volume of 30 μl buffer (Hepes 50 mM pH 7.5, $MgCl_2$ 3 mM, $MnCl_2$ 3 mM, DTT 1 mM, $NaVO_3$ 3 μM, + 0.2 mg/ml BSA) were added to each well of a 96 U bottom.

After incubation for 20 min at room temperature, the reaction was stopped by addition of 100 µl PBS buffer containing 32 mM EDTA, 500 µM cold ATP, 0.1% Triton X100 and 10mg/ml streptavidin coated SPA beads. After 20 min incubation, 110 µL of suspension were withdrawn and transferred into 96-well OPTIPLATEs containing 100 µl of 5M CsCl. After 4 hours, the plates were read for 2 min in a Packard TOP-Count radioactivity reader.

IC50 determination: see above

Inhibition assay of IGF1-R activity

The inhibition assay of IGF1-R activity is performed according to the following protocol.

Enzyme activation: IGF1-R must be activated by auto-phosphorylation before starting the experiment. Just prior to the assay, a concentrated enzyme solution (694 nM) is incubated for half a hour at 28°C in the presence of 100 µM ATP and then brought to the working dilution in the indicated buffer.

Kinase reaction: 10 µM biotinylated IRS1 peptide (PRIMM) substrate, 0-20 µM inhibitor, 6 µM ATP, 1 microCi ^{33}P-ATP, and 6 nM GST-IGF1-R (pre-incubated for 30 min at room temperature with cold 60 µM cold ATP) in a final volume of 30 µl buffer (50 mM HEPES pH 7.9, 3 mM $MnCl_2$, 1 mM DTT, 3 µM $NaVO_3$) were added to each well of a 96 U bottom well plate. After incubation for 35 min at room temperature, the reaction was stopped by addition of 100 µl PBS buffer containing 32 mM EDTA, 500 µM cold ATP, 0.1% Triton X100 and 10mg/ml streptavidin coated SPA beads. After 20 min incubation, 110 µL of suspension were withdrawn and transferred into 96-well OPTIPLATEs containing 100 µl of 5M CsCl. After 4 hours, the plates were read for 2 min in a Packard TOP-Count radioactivity reader.

Inhibition assay of Aurora-2 activity

Kinase reaction: 8 µM biotinylated peptide (4 repeats of LRRWSLG), 10 µM ATP (0.5 uCi $P^{33}\gamma$-ATP), 7.5 ng Aurora 2, inhibitor in a final volume of 30 µl buffer (HEPES 50 mM pH 7.0, $MgCl_2$ 10 mM, 1 mM DTT, 0.2 mg/ml BSA, 3 µM orthovanadate) were added to each well of a 96 U bottom well plate. After 60 minutes

at room temperature incubation, reaction was stopped and biotinylated peptide captured by adding 100 µl of bead suspension.

Stratification: 100 µl of CsCl2 5 M were added to each well and let stand 4 hour before radioactivity was counted in the Top-Count instrument.

IC50 determination: see above

Inhibition assay of Cdc7/dbf4 activity

The inhibition assay of Cdc7/dbf4 activity is performed according to the following protocol.

The Biotin-MCM2 substrate is trans-phosphorylated by the Cdc7/Dbf4 complex in the presence of ATP traced with γ^{33}-ATP. The phosphorylated Biotin-MCM2 substrate is then captured by Streptavidin-coated SPA beads and the extent of phosphorylation evaluated by β counting.

The inhibition assay of Cdc7/dbf4 activity was performed in 96 wells plate according to the following protocol.

To each well of the plate were added:

- 10 µl substrate (biotinylated MCM2, 6 µM final concentration)
- 10 µl enzyme (Cdc7/Dbf4, 17.9 nM final concentration)
- 10 µl test compound (12 increasing concentrations in the nM to µM range to generate a dose-response curve)
- 10 µl of a mixture of cold ATP (2 µM final concentration) and radioactive ATP (1/5000 molar ratio with cold ATP) was then used to start the reaction which was allowed to take place at 37°C.

Substrate, enzyme and ATP were diluted in 50 mM HEPES pH 7.9 containing 15 mM $MgCl_2$, 2 mM DTT, 3 µM $NaVO_3$, 2mM glycerophosphate and 0.2mg/ml BSA. The solvent for test compounds also contained 10% DMSO.

After incubation for 60 minutes, the reaction was stopped by adding to each well 100 µl of PBS pH 7.4 containing 50 mM EDTA, 1 mM cold ATP, 0.1% Triton X100 and 10 mg/ml streptavidin coated SPA beads.

After 20 min incubation, 110 µL of suspension were withdrawn and transferred into 96-well OPTIPLATEs containing 100 µl of 5M CsCl. After 4 hours, the plates were read for 2 min in a Packard TOP-Count radioactivity reader.

IC50 determination: see above.

The compounds of formula (I) of the present invention, suitable for administration to a mammal, e.g. to humans, can be administered by the usual routes and the dosage level depends upon the age, weight, conditions of the patient and the administration route.

For example, a suitable dosage adopted for oral administration of a compound of formula (I) may range from about 10 to about 500 mg pro dose, from 1 to 5 times daily.

The compounds of the invention can be administered in a variety of dosage forms, e.g. orally, in the form of tablets, capsules, sugar or film coated tablets, liquid solutions or suspensions; rectally in the form of suppositories; parenterally, e.g. intramuscularly, or by intravenous and/or intrathecal and/or intraspinal injection or infusion.

In addition, the compounds of the invention can be administered either as single agents or, alternatively, in combination with known anticancer treatments such as radiation therapy or chemotherapy regimen in combination with cytostatic or cytotoxic agents, antibiotic-type agents, alkylating agents, antimetabolite agents, hormonal agents, immunological agents, interferon-type agents, cyclooxygenase inhibitors (e.g. COX-2 inhibitors), metallomatrixprotease inhibitors, telomerase inhibitors, tyrosine kinase inhibitors, anti-growth factor receptor agents, anti-HER agents, anti-EGFR agents, anti-angiogenesis agents, farnesyl transferase inhibitors, ras-raf signal transduction pathway inhibitors, cell cycle inhibitors, other cdks inhibitors, tubulin binding agents, topoisomerase I inhibitors, topoisomerase II inhibitors, and the like.

As an example, the compounds of the invention can be administered in combination with one or more chemotherapeutic agents such as, for instance, exemestane, formestane, anastrozole, letrozole, fadrozole, taxane, taxane derivatives, encapsulated taxanes, CPT-11, camptothecin derivatives, anthracycline glycosides, e.g., doxorubicin, idarubicin, epirubicin, etoposide, navelbine, vinblastine, carboplatin, cisplatin, estramustine, celecoxib, tamoxifen, raloxifen, Sugen SU-5416, Sugen SU-6668, Herceptin, and the like, optionally within liposomal formulations thereof.

If formulated as a fixed dose, such combination products employ the compounds of this invention within the dosage range described above and the other pharmaceutically active agent within the approved dosage range.

Compounds of formula (I) may be used sequentially with known anticancer agents when a combination formulation is inappropriate.

It is therefore a further object of the invention a product or kit comprising the compound of formula (I) of the invention and one or more chemotherapeutic agents for simultaneous, separate or sequential use in anticancer therapy or for the treatment of cell proliferative disorders.

The present invention also includes pharmaceutical compositions comprising an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable excipient, carrier or diluent.

The pharmaceutical compositions containing the compounds of the invention are usually prepared following conventional methods and are administered in a pharmaceutically suitable form.

For example, the solid oral forms may contain, together with the active compound, diluents, e.g. lactose, dextrose, saccharose, sucrose, cellulose, corn starch or potato starch; lubricants, e.g. silica, talc, stearic, magnesium or calcium stearate, and/or polyethylene glycols; binding agents, e.g. starches, arabic gum, gelatine, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disaggregating agents, e.g. a starch, alginic, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. Said pharmaceutical preparations may be manufactured in known manner, for example, by means of mixing, granulating, tabletting, sugar-coating, or film-coating processes.

The liquid dispersions for oral administration may be e.g. syrups, emulsions and suspensions.

The syrups may contain as carrier, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol.

The suspensions and the emulsions may contain as carrier, for example, a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol.

The suspension or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and, if desired, a suitable amount of lidocaine hydrochloride. The solutions for intravenous injections or infusions may contain as carrier, for example, sterile water or preferably they may be in the form of sterile, aqueous, isotonic saline solutions or they may contain as a carrier propylene glycol.

The suppositories may contain together with the active compound a pharmaceutically acceptable carrier, e.g. cocoa butter, polyethylene glycol, a polyoxyethylene sorbitan fatty ester surfactant or lecithin.

The following examples illustrates the invention without limiting it.

Example 1

Preparation of 5-tert-butyloxycarbonyl-1-ethoxycarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine,

(R_a=R_b=R_c=R_d= H, R=H, R_1=t-butyloxycarbonyl(BOC), R_2= ethoxycarbonyl).

A solution of 3-amino-5-tert-butyloxycarbonyl-1-ethoxycarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine (0.4g, 1.35 mmol) in dry tetrahydrofurane (10ml) was added dropwise to a solution of isoamylnitrite (0.32ml, 2.36mmol) in dry tetrahydrofurane (2ml) maintained at reflux. The resulting solution was stirred at reflux for 4 hours, and then cooled to room temperature. After removal of the solvent under vacuum, the crude material was purified by flash chromatography on silica gel using n-hexane÷ethyl acetate 90÷10; 70÷30. The title compound was obtained as a light yellow oil .

Operating in an analogous way, the following compound is also obtained:

5-tert-butyloxycarbonyl-2-ethoxycarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine.

Example 2

Preparation of 5-tert-butyloxycarbonyl-1(2)H- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=H, R_1=t-butyloxycarbonyl(BOC), R_2= H).

5-tert-butyloxycarbonyl-1-ethoxycarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine (1.5g,) was treated with a solution of 10% triethylamine in methanol (74 ml) at room temperature for about 20 hours. After removal of the solvents under vacuum, the crude material was dissolved with chloroform (30ml) and washed with water (20mlx2), brine (20ml), dried over sodium sulphate, filtered and evaporated to dryness. The title compound was obtained as a beige powder (1g).

Operating in an analogous way, the following compound was obtained:

3-iodo-5-isopropylaminocarbonyl-1(2)H- pyrazolo [4,3-c] 4,5,6,7-tetrahydro pyridine, (R_a=R_b=R_c=R_d= H, R=I, R_1=3-isopropylaminocarbonyl, R_2= H).

Example 3

Preparation of 1-ethoxycarbonyl-5-(3-methylbutanoyl)-3-iodo- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=I, R_1=3-methylbutanoyl, R_2= 1-ethoxycarbonyl).

A solution of 5-tert-butyloxycarbonyl-1-ethoxycarbonyl-3-iodo- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine (0.7g) in dichloromethane (40ml) was treated with trifluoroacetic acid (9ml) at room temperature for about 4 hours. After removal of the solvents, the crude salt was dissolved with dry tetrahydrofurane (40ml) and added with diisopropyl ethyl amine (1.47ml) and isovaleroyl chloride (0.23ml) diluted with dry tetrahydrofurane (2ml). The reaction mixture was stirred at room temperature for about 20 hours; the solvent was evaporated under vacuum and the crude material was dissolved with dichloromethane (25ml), washed with water (15ml), brine (15ml), dried over sodium sulphate, filtered and dried under vacuum to yield the title compound as a light brown solid which was used without any further purification (0.65g).

Operating in an analogous way, the following compound was also obtained:

1-ethoxycarbonyl-3-iodo-5-isopropylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine.

Example 4

Preparation of 5-tert-butyloxycarbonyl-1-ethoxycarbonyl-3-iodo- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Iodo, R_1=t-butyloxycarbonyl(BOC), R_2= ethoxycarbonyl).

Isoamyl nitrite (0.89 ml, 6.64mmol) was slowly added to a mixture of Iodine (1.01 g, 3.98 mmol) in 10 mL of anhydrous dichloromethane, at +22°C. To this dark mixture a solution of 5-tert-butyloxycarbonyl-1-ethoxycarbonyl-3-amino- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine (1.03 g, 3.32 mmol) in 28 mL of dichloromethane was added dropwise over 40 min at +22°C. The internal temperature rose to +28°C and gas was evolved during the addition. After 1 hour stirring at room temperature, the reaction mixture was slowly poured in 40 ml of 10% sodium metabisulfite. The phases were separated and the aqueous was extracted twice with dichloromethane. The combined extracts were dried over anhydrous sodium sulfate and the solvent evaporated under vacuum. This raw material was purified by flash chromatography eluting with 20:80 EtOAc/cyclohexane. A white solid (0.75 g) was obtained. (54% yield). m.p. 178-181°C. ^{1}H-NMR(DMSO-d_6) δ ppm: 4.38(q,2H);4.11(m,2H); 3.57(m, 2H); 2.90(m, 2H); 1.40(s,9H); 1.30(t,3H).

Example 5

Preparation of 5-tert-butyloxycarbonyl-3-iodo- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Iodo, R_1=t-butyloxycarbonyl(BOC), R2= H).

1-ethoxycarbonyl-3-iodo-5-tert-butyloxycarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine (250 mg) was stirred with a mixture of MeOH (2 ml) and triethylamine (0.5 ml) at room temperature for about 30 min. The solvents were evaporated and the compound was dried under vacuum. White solid (200 mg).

Example 6

Preparation of 5-tert-butyloxycarbonyl-3-phenyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine,

(R_a=R_b=R_c=R_d= H, R=Phenyl, R_1=t-butyloxycarbonyl(BOC), R_2= H).

A mixture of 5-tert-butyloxycarbonyl-1-ethoxycarbonyl-3-iodo- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine (60 mg), phenylboronic acid (22 mg), potassium carbonate (31 mg), triethylamine (ml 0.03) and palladiumdichloride-diphenylphosphine (8mg, 7%) in dioxane/water 10/1 (2ml) was heated under Argon atmosphere at 80°C for about 3 hours. The mixture was diluted with ethyl acetate (8ml), washed with water (5ml), brine (5ml), dried over sodium sulphate, filtered and evaporated to dryness. The crude

material was purified by flash chromatography, using Ethylacetate/exane as eluent to yield the title compound as a light yellow solid (30mg).

Example 7

Preparation of 5-acetyl-3-phenyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenyl, R_1=Acetyl, R_2= H).

A solution of 5-tert-butyloxycarbonyl-3-phenyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine (90 mg) in dichloromethane (3.5ml) was treated with trifluoroacetic acid (0.5ml), at room temperature for about 4 hours. After removal of the solvents, the crude salt was dissolved with dry diclhorometane (5ml) and added with diisopropylethylamine (0.32 ml) and acetyl chloride (0.07ml). The reaction mixture was stirred at room temperature for about 2 hours; the crude material was diluted with dichloromethane (25ml), washed with water (15ml), brine (15ml), dried over sodium sulphate, filtered and dried under vacuum. The crude was suspended in a solution of sodium bicarbonate and stirred at room temperature for about 3 hours, then extracted with ethylacetate to yield the title compound as a light brown solid (40 mg).

Example 8

Preparation of 5-tert-butyloxycarbonyl-3-iodo-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Iodo, R_1=t-butyloxycarbonyl(BOC), R_2= polystyrenemethylaminocarbonyl).

The isocyanate methylpolystyrene resin (1.14 g, 1,71 mmol) was swelled with 15 ml of dichloromethane, and a solution of 5-tert-butyloxycarbonyl-3-iodo- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine (400 mg) in 3 ml of dimethylformamide was added.

The mixture was stirred at room temperature for about 24 hours; after filtration, the resin was washed with dichlorometane (2 x 20 ml), MeoH (2 x 20 ml), dimethylformamide (2 x 20 ml) and dichloromethane (3 x 20 ml).

The resin was dried under vacuum.

Example 9

Preparation of

5-tert-butyloxycarbonyl-3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenyl, R_1=t-butyloxycarbonyl(BOC), R_2= polystyrenemethylaminocarbonyl).

To a suspension of 5-tert-butyloxycarbonyl-3-iodo-1-polystyrenemethylaminocarbonyl-pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine (117 mg) in dioxane/water 10/1 (3 ml), phenylboronic acid (108 mg), potassium carbonate (171 mg), triethylamine (0.18 ml, 0.8 mmol) and palladiumdichloride diphenylphosphine (25 mg, 20%) were added.

The mixture was stirred at 80°C for about 8 hours; after filtration, the resin was washed with dichlorometane (2 x 20 ml), MeoH (2 x 20 ml), dimethylformamide (2 x 20 ml) and dichloromethane (3 x 20 ml).

The resin was dried under vacuum.

Operating in an analogous way, using a suitable boronic acid, the following compounds are also obtained:

5-tert-butyloxycarbonyl-3-(4-phenoxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-phenoxy-phenyl, R_1=t-butyloxycarbonyl(BOC), R_2= polystyrenemethylaminocarbonyl);

3-(4-benzyloxy-phenyl)-5-tert-butyloxycarbonyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-benzyloxy-phenyl, R_1=t-butyloxycarbonyl(BOC), R_2= polystyrenemethylaminocarbonyl);

5-tert-butyloxycarbonyl-3-(5-chloro-thiphen-2-yl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=5-chloro-thiphen-2-yl, R_1=t-butyloxycarbonyl(BOC), R_2= polystyrenemethylaminocarbonyl);

5-tert-butyloxycarbonyl-3-(4-methoxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-methoxy-phenyl, R_1= t-butyloxycarbonyl(BOC), R_2= polystyrenemethylaminocarbonyl) and

5-tert-butyloxycarbonyl-3-(4-dimethylamino-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-dimethylamino-phenyl, R_1=t-butyloxycarbonyl(BOC), R_2= polystyrenemethylaminocarbonyl).

Example 10

Preparation of 5-tert-butyloxycarbonyl-3-phenylethynyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenylethynyl, R_1=t-butyloxycarbonyl(BOC), R_2= polystyrenemethylaminocarbonyl).

To a suspension of 5-tert-butyloxycarbonyl-3-iodo-1-polystyrenemethylaminocarbonyl-pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine (200 mg) in dioxane (2 ml), phenylethyne (0.23 ml), CuI (20 mg, 50%), triethylamine (0.12 ml) and palladiumdichloride diphenylphosphine (29 mg, 20%) were added.

The mixture was stirred at 80°C for about 8 hours; after filtration, the resin was washed with dichlorometane (2 x 20 ml), MeoH (2 x 20 ml), dimethylformamide (2 x 20 ml) and with dichloromethane (3 x 20 ml).

The resin was dried under vacuum.

Example 11

Preparation of 3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenyl, R_1=H, R_2= polystyrenemethylaminocarbonyl).

To 5-tert-butyloxycarbonyl-3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine swelled in dichloromethane (5 ml) trifluoroacetic acid (1 ml) was added.

The mixture was stirred at room temperature for about 4 hours, after filtration, the resin was washed with dichlorometane (2 x 20 ml), MeoH (2 x 20 ml), dimethylformamide (2 x 20 ml) and dichloromethane (3 x 20 ml).

The resin was dried under vacuum.

Operating in an analogous way, the following compounds are also obtained:

3-(4-phenoxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenyl, R_1=H, R_2= polystyrenemethylaminocarbonyl);

3-(4-benzyloxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Benzyloxyphenyl, R_1=H, R_2= polystyrenemethylaminocarbonyl);

3-(5-chloro-thiophen-2-yl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=5-Chloro-thiophen-2-yl, R_1=H, R_2= polystyrenemethylaminocarbonyl);

3-(4-methoxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Methoxyphenyl, R_1=H, R_2= polystyrenemethylaminocarbonyl);

3-(4-dimethylamino-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Dimethylaminophenyl, R_1=H, R_2= polystyrenemethylaminocarbonyl) and

3-phenylethynyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenylethynyl, R_1=H, R_2= polystyrenemethylaminocarbonyl).

Example 12

Preparation of 5-acetyl-3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenyl, R_1=Acetyl, R_2= polystyrenemethylaminocarbonyl).

To 3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine swelled in dichloromethane (5 ml) diisopropylethylamine (0.21 ml) and acetylchloride (0.06 ml) were added.

The mixture was stirred at room temperature for about 24 hours; after filtration, the resin was washed with dichlorometane (2 x 20 ml), MeoH (2 x 20 ml), dimethylformamide (2 x 20 ml) and dichloromethane (3 x 20 ml). The resin was dried under vacuum.

Operating in an analogous way, the following compounds are also obtained:

5-acetyl-3-(4-phenoxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Phenoxyphenyl, R_1=Acetyl, R_2= polystyrenemethylaminocarbonyl);

5-acetyl-3-(4-benzyloxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Benzyloxyphenyl, R_1=Acetyl,

R_2= polystyrenemethylaminocarbonyl);

5-acetyl-3-(5-chloro-thiphen-2-yl) -1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=5-chloro-thiophen-2-yl, R_1=Acetyl, R_2= polystyrenemethylaminocarbonyl);

5-acetyl-3-(4-methoxy-phenyl)- 1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Methoxyoxyphenyl, R_1=Acetyl, R_2= polystyrenemethylaminocarbonyl);

5-acetyl-3-(4-dimethylamino-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Dimethylamino-phenyl R_1=Acetyl, R_2= polystyrenemethylaminocarbonyl) and

5-acetyl-3-phenylethynyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenylethynyl, R_1=Acetyl, R_2= polystyrenemethylaminocarbonyl).

Example 13

Preparation of 5-isopropylaminocarbonyl-3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenyl, R_1=Isopropylaminocarbonyl, R_2= polystyrenemethylaminocarbonyl).

To 3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine swelled in dichloromethane (5 ml) isopropylisocyanate (0.09 ml) was added.

The mixture was stirred at room temperature for about 24 hours; after filtration, the resin was washed with dichlorometane (2 x 20 ml), MeoH (2 x 20 ml), dimethylformamide (2 x 20 ml) and dichloromethane (3 x 20 ml). The resin was dried under vacuum.

Operating in an analogous way, the following compounds are also obtained:

5-isopropylaminocarbonyl-3-(4-phenoxy-phenyl)-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Phenoxyphenyl, R_1=Isopropylaminocarbonyl, R_2= polystyrenemethylaminocarbonyl);

3-(4-benzyloxy-phenyl)-5-isopropylaminocarbonyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Benzyloxyphenyl, R_1=Isopropylaminocarbonyl, R_2= polystyrenemethylaminocarbonyl);

3-(5-chloro-thiphen-2-yl)-5-isopropylaminocarbonyl -1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=5-Chloro-thiphen-2-yl, R_1=Isopropylaminocarbonyl, R_2= polystyrenemethylaminocarbonyl);

5-isopropylaminocarbonyl -3-(4-methoxy-phenyl)- 1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Methoxy-phenyl, R_1=Isopropylaminocarbonyl, R_2= polystyrenemethylaminocarbonyl);

3-(4-dimethylamino-phenyl)-5-isopropylaminocarbonyl -1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Dimethylamino-phenyl, R_1=Isopropylaminocarbonyl, R_2= polystyrenemethylaminocarbonyl) and

5-isopropylaminocarbonyl -3-phenylethynyl- 1-polystyrenemethylaminocarbonyl-pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenylethynyl, R_1=Isopropylaminocarbonyl, R_2= polystyrenemethylaminocarbonyl).

Example 14

Preparation of 5-acetyl-3-phenyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenyl, R_1=Acetyl, R_2= H).

To 5-acetyl-3-phenyl-1-polystyrenemethylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine (200 mg) swelled in dioxane (3 ml), sodium hydroxide (35% in water) was added (0.4 ml) and the mixture was stirred at 40°C for about 90 hours.

After neutralization of the solution, the mixture was filtered and the desired product was dried under vacuum: white solid (40 mg).

Operating in an analogous way, the following compounds were also obtained.

5-isopropylaminocarbonyl-3-phenyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenyl, R_1=Isopropylaminocarbonyl, R_2= H);

5-acetyl-3-(4-phenoxy-phenyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Phenoxy-phenyl, R_1=Acetyl, R_2= H);

5-isopropylaminocarbonyl-3-(4-phenoxy-phenyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Phenoxy-phenyl, R_1=Isopropylaminocarbonyl, R_2= H);

5-acetyl-3-(4-benzyloxy-phenyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Benzyloxy-phenyl, R_1=Acetyl, R_2= H);

3-(4-benzyloxy-phenyl)-5-isopropylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Benzyloxy-phenyl, R_1=Isopropylaminocarbonyl, R_2= H);

5-acetyl-3-(5-chloro-thiophen-2-yl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=5-Chloro-thiophen-2-yl, R_1=Acetyl, R_2= H);

3-(5-chloro-thiophen-2-yl)-5-isopropylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=5-Chloro-thiophen-2-yl, R_1=Isopropylaminocarbonyl, R_2= H);

5-acetyl-3-(4-methoxy-phenyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Methoxy-phenyl, R_1=Acetyl, R_2= H);

5-isopropylaminocarbonyl-3-(4-methoxy-phenyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Methoxy-phenyl, R_1= Isopropylaminocarbonyl, R_2= H);

5-acetyl-3-(4-dimethylamino-phenyl)- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Dimethylamino-phenyl, R_1=Acetyl, R_2= H);

3-(4-dimethylamino-phenyl)-5-isopropylaminocarbonyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=4-Dimethylamino-phenyl, R_1= Isopropylaminocarbonyl, R_2= H).

5-acetyl-3-phenylethynyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d=H, R=Phenylethynyl, R_1=Acetyl, R_2= H);

5-isopropylaminocarbonyl-3-phenylethynyl- pyrazolo [4,3-c] 4,5,6,7-tetrahydropyridine, (R_a=R_b=R_c=R_d= H, R=Phenylethynyl, R_1= Isopropylaminocarbonyl, R_2= H);